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PREDICTION OF HUMAN SYSTEMIC, BIOLOGICALLY RELEVANT PHARMACOKINETIC PROPERTIES BASED ON PHYSICOCHEMICAL PROPERTIES OF CALCIUM CHANNEL BLOCKERS

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A thesis submitted in partial fulfillment of the requirements for the degree of Master of Sciences at Virginia Commonwealth University.

By

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List of Abbreviations

ADME	Absorption, distribution, metabolism and elimination
AUC	Area under the plasma concentration-time profile curve
AUC_{∞}	Area under the curve from zero to infinity
a, b	Hybrid rate constants
A, B	Exponential coefficients ($Cpt = Ae^{-\alpha t} + Be^{-\beta t}$)
α, β	Macro-rate constants
CCB	Calcium channel blockers
β-ARL	Beta-adrenergic receptor ligand
BW	Body weight
$CL_{extrahepatic}$	Extrahepatic clearance
CL_{hep}	Hepatic clearance
CL_{int}	Intrinsic hepatic clearance
clogP	Calculated logarithm of octanol-water partition coefficient
CL_{nonren}	Nonrenal clearance
CL_{nonren}^{u}	Unbound nonrenal clearance
CL_{ren}	Renal clearance
CL_{ren}^{u}	Unbound renal clearance
CL_{tot}	Total body clearance
CL_{tot}^{u}	Unbound total body clearance
c_{max}	Observed maximal concentration
DHP	1, 4-dihydropyridines
ER_{hep}	Hepatic extraction ratio
f_e	Fraction of dose excreted unchanged in urine after intravenous administration
F_{oral}	Oral bioavailability
f_u	Fraction unbound in plasma
GFR	Glomerular filtration rate
GIT	Gastrointestinal tract

HBA	Number of hydrogen bond donors
HBD	Number of hydrogen bond acceptors
HER	High hepatic extraction ratio
IV	Intravenous administration
k_{10}	Elimination rate constant
k_{12}	First-order transfer rate constant from central to peripheral compartment
k_{21}	First-order transfer rate constant from peripheral to central compartment
LBF	Liver blood flow
LER	Low extraction ratio
LogD	Logarithm of distribution coefficient
$LogD_{7.4}$	Logarithm of octanol-buffer @ pH 7.4 distribution coefficient
LogP	Logarithm of octanol-water partition coefficient
LLOQ	Lower limit of quantitation
MRT_{sys}	Systemic mean residence time
MW	Molecular weight
MV	Molar volume
nRot	Number of rotatable bonds
PC	Physicochemical
PD	Pharmacodynamics
PK	Pharmacokinetics
PPB	Plasma protein binding
PSA	Polar surface area
QSAR	Quantitative structure-activity relationship
RBF	Renal blood flow
Vd	Volume of distribution
Vd_{β}	Terminal phase volume of distribution
Vd_{cc}	Volume of distribution of central compartment
Vd_{ss}	Volume of distribution at steady-state
Vd_{ss}/F_{oral}	Apparent volume of distribution at steady-state
Vd_{ss}^{u}	Unbound volume of distribution at steady-state

Abstract

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This research explored quantitative relationships (QSPKR) between different molecular descriptors and pertinent, systemic PK properties for 14 calcium channel blockers (CCB). Physicochemical properties (PC) such as molecular weight (MW), molar volume (MV), calculated logP (clogP), pK_a, calculated $logD_{7.4}$ (clogD), % ionized at pH 6.3 and pH 7.4, hydrogen bond donors (HBD), hydrogen bond acceptors (HBA), and number of rotatable bonds (nRot) were chosen as possible predictor variables for systemic PK properties for CCB, obtained from pertinent literature, assessing the PK of CCB after intravenous administration to healthy humans.

All PC properties and molecular descriptors were computed using ACD-solubility/DB 12.01. Total body clearance (CL_{tot}), steady-state volume of distribution (Vd_{ss}), total area under the plasma concentration-time profile (AUC_{oo}), terminal half-life ($t_{1/2}$), and fraction of drug excreted unchanged

in urine (f_e), if available, were obtained or derived from original references, exclusively from IV studies that administered CCB to healthy human volunteers. Several articles focused on drug interactions with grapefruit juice or the impact of renal/hepatic dysfunction, and in such cases, data from the healthy control group were used. Each study was evaluated for study design, PK sampling schedule, bioanalytical and PK analysis methods before inclusion into the final database.

The assumption of linear systemic PK was verified by assessing AUC_{oo} versus (IV) dose. Plasma protein binding information was collected from *in-vitro* experiments to obtain the fraction unbound in plasma (f_u). Unbound volume of distribution at a steady state (Vd_{ss}^{u}), unbound total (CL_{tot}^{u}), renal (CL_{ren}^{u}), and non-renal clearance (CL_{nonren}^{u}) were estimated and compared with the relevant physiological references for Vd_{ss}^{u} (plasma volume, blood volume, extracellular and intracellular spaces, total body water and body weight) and for the unbound clearances (liver blood flow, renal plasma flow, and glomerular filtration rate, GFR). Final PK property values were obtained by averaging across available studies. The distribution of both PC and PK properties were evaluated, and correlation matrices amongst PC properties were constructed to assess for collinearity. If two PC descriptors were found to be collinear, i.e. r, ≥ 0.8, only one of them was used in the final univariate analysis. Finally, univariate linear regression of all PK variables versus each molecular descriptor was performed; any relationship with $p<0.05$ and $r^2 \geq 0.30$ was considered to be statistically significant.

The PC properties of the final 14 CCB were reasonably normally distributed with few exceptions. Overall, CCBs are small (MW range of 316-496 Da), basic and lipophilic ($logD_{7.4}$ range of 1.5-5.1) molecules. On the other hand, for the PK properties, the distributions were found to be skewed with high standard deviations. Thus, all PK variables (except f_u) were log-transformed. Although CCB are mostly highly plasma protein bound (f_u range of 0.2-20%), they are characterized

by extensive extravascular tissue distribution (Vd_{ss} range of 0.6-20.4 l/kg) and high, mainly metabolic, clearance (CL_{tot} range of 3.7-131.7 ml/min/kg). Clevidipine is the only CCB undergoing extensive, extra-hepatic ester hydrolysis, responsible for the highest CL_{tot} value. Urinary excretion for CCB is negligible. Amlodipine is a PK outlier due to its high Vd_{ss} (20.4 l/kg) and low CL_{tot} (6.9 ml/min/kg, due to low hepatic extraction) with f_u of 2%. Therefore, the final QSPKR analysis was performed including, as well as excluding amlodipine. Excluding amlodipine, the relationship between f_u and $logD_{7.4}$ was negative and significant (r^2 of 0.4, n=12). The relationships between CL_{tot}^u, CL_{nonren}^u and CL_{ren}^u and $logD_{7.4}$ were found to be positive and significant (r^2 between 0.6-0.7, n=3-12); none of the other PC variables affected any of the clearance terms. Although the relationship between Vd_{ss}^u and $logD_{7.4}$ was not significant (r^2 of 0.25, n=12), it showed the expected positive slope. In fact, after removing bepridil (the remaining outlier in Vd_{ss}^u), the relationship with $logD_{7.4}$ became statistically significant (r^2=0.46, n=11).

The QSPKR obtained in this study for CCB, with $logD_{7.4}$ being the main PC determinant for systemic PK properties, were similar to those previously reported for opioids, β-adrenergic receptor ligands and benzodiazepines. However, slope estimates for the relationships of CL_{nonren}^u and CL_{tot}^u as a function of $logD_{7.4}$ for CCB were higher compared to these previously studied compounds, which showed higher sensitivity, most likely as a result of their higher lipophilicity. Overall, lipophilicity measured as $logD_{7.4}$ was found to be a statistically significant and plausible PC determinant for the biologically relevant systemic PK properties for CCB and other classes of drugs.

1 INTRODUCTION

1.1.1 Background

The pharmaceutical industry faces many challenges in discovering and developing new drugs for clinical use, costing millions of dollar every year; these costs are expected to increase. Dimasi et al.[1] estimated the cost of development of a single new chemical entity approaching $1 billion. Thousands of chemical compounds are tested every year, but only a few drugs reach the market, which makes the development of new drugs expensive and time consuming. Thus, one of the major challenges in discovery and development is to find a way to reduce the costs. The costs could be reduced if pharmaceutical scientists were able to predict or identify undesirable compounds at an early stage of drug discovery/development. Therefore, many studies have been performed to understand possible reasons behind drug development failures. Lipper et al.[2] found that 63% of all pre-clinical compounds failed due to poor pharmacokinetics (PK) and/or drug toxicity. As a result, the early prediction of human PK is very important, and most companies and agencies have their own approaches, which they believe is the best way to save time and money. Inter-species pharmacokinetic allometric scaling, physiologically-based PK modeling, *in-vitro-in-vivo* extrapolation and quantitative structure pharmacokinetic relationships (QSPKR) are used for the prediction of PK in the early stage of development. Each method has its own advantages and drawbacks. For example, allometric PK scaling has been used successfully in the prediction of human volume of distribution (Vd), however, it may not be useful in predicting total body clearance (CL_{tot}), especially when a high fraction of clearance is via drug metabolism.[3] QSPKR models are mathematical equations relating quantitative information obtained from the chemical molecular structure to their biological activity and PK property. The aim of this study is to discover the most important PC properties that are able to predict human PK properties of the available CCB.

Calcium (Ca^{+2}) plays a major role in various cellular functions such as muscle contraction and relaxation, as well as cellular secretion.[4, 5] CCB interact with the L-type voltage-gated calcium channels in cell membranes to modulate the intracellular concentration of calcium.[4] Contractile myofilaments (such as those in cardiac and smooth muscles) are activated when the intracellular concentration of Ca^{+2} increases. Excitation–contraction coupling in smooth and cardiac muscles is very sensitive to these changes. Therefore, the myocardial and smooth muscle contractility depends on the amount of Ca^{+2} coming from the extracellular space. Inhibition of Ca^{+2} influxes to cardiac muscles causes negative inotropism, while, in vascular smooth muscle, it causes vasodilation and hypotension. As a result, these drugs can be utilized to treat arterial hypertension, angina pectoris, congestive heart failure and other cardiovascular diseases.[4, 5] CCB are among the most widely used drugs in the treatments of hypertension and cardiovascular disease that are alone or in some time combined with other antihypertensive drugs. However, there are tremendous PK differences in term of their clinical use of these compounds. Also there are metabolism differences between certain groups of patients, such as in case of nifedipine metabolism.[6]

1.1.2 History of calcium channel blockers (CCB)

Fleckenstein first discovered CCB in 1963 when he was experimenting with two newly synthesized compounds. He reported that these two drugs (verapamil and prenylamine) were able to diminish the cardiac contractile force without a major change in action potential, and could be easy neutralized by administration of calcium. Thus, he realized that this effect was most probably due to interfering with calcium function during the excitation–contraction coupling. Fleckenstein chose to study verapamil further because it was more potent than prenylamine.[5] In 1966, Bender et al.[7] conducted a study to examine the effects of verapamil in humans as antiarrhythmic and

antihypertensive drug. Fleckenstein discovered nifedipine in 1970, and he introduced the term "calcium antagonist" to describe these compounds. CCB were introduced in the market in the late 1970s and early 1980s namely nifedipine, diltiazem and verapamil. Gallopamil, the methoxy derivative of verapamil, was discovered in 1986 and had a higher potency than verapamil. In fact, these drugs do not share the same pharmacophore, but they share the same mechanism of action, blocking the entrance of calcium into cardiac and smooth muscle. After these discoveries, many new CCB were discovered.[9] Fleckenstein first classified these drugs as calcium antagonist group "A", able to selectively suppress the slow calcium current and not suppress magnesium current. On the other hand, those drugs that affect both calcium and magnesium current were classified as group "B". More CCB were discovered and the classification was changed to reflect these new discoveries.[9] Today, CCB are classified broadly into three classes based on their preferential site of action (arterial vessels and/or the heart), administration frequency, duration of action and chemical structure, namely phenylalkylamines (verapamil), 1,4-dihydropyridines (nifedipine, amlodipine, clevidipine, felodipine, isradipine, nimodipine and nicardipine), and benzothiazepines (diltiazem).[4, 10] Most of CCB on the market belong to dihydropyridine group, and within subclasses, compounds are further separated into first, second and third generation based on their PK and PD properties. First generation CCB had many side effects and the second generation came to overcome these side effects. Thus, the third generation CCB has the best PK and PD profiles such as long half-life and higher tissue selectivity. However, in some cases like clevidipine (third generation CCB), was design to have an ultra-short half-life to use it as antihypertensive medication during surgery. Thus, it depends on the indication.[10]

1.1.3 Chemical structures of CCB

Many inorganic cations like manganese and lanthanum can easily interfere with calcium in the binding sites, but this binding is nonspecific and nonselective, thus, these cations cannot be used for treatments as antihypertensive or antiarrhythmic drugs.[11] Compounds that are more specific to interact with calcium and more selective to smooth and/or cardiac muscle can be used as CCB for the treatments of hypertension and cardiovascular disorders. Since 1, 4-dihydropyridine CCB are the largest group among the three principal classes of drugs, the focus will be on this class. They have a dihydropyridine ring (dialkyl 4-aryl-1, 4 dihydropyridine-3, 5 dicarboxylates) as shown in Figure 1.1.[10]

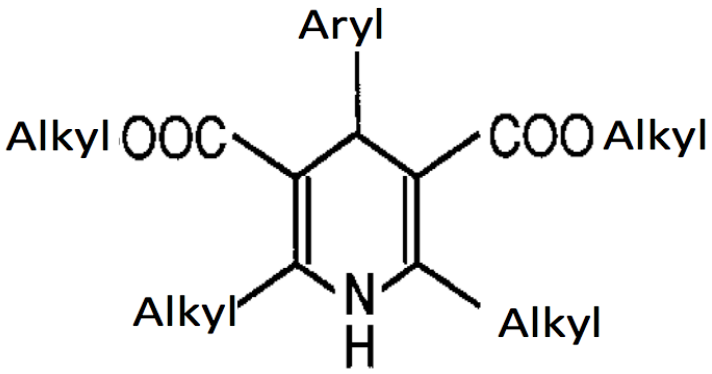


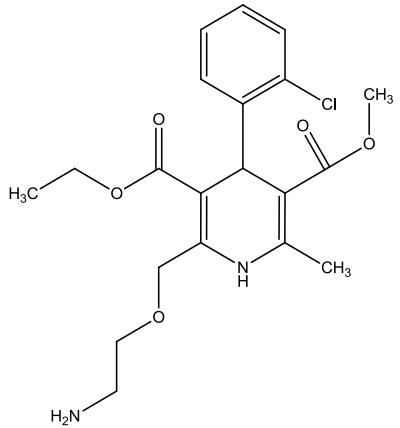
Figure 1.1 General structure of 1,4 dihydropyridine

The first development in the discovery of this class started in 1951 when khellin was discovered in the Langendorff heart preparation. The structure of khellin was studied and modified by Bayer Chemical Research Laboratories, which lead to the discovery of 1, 4 dihydropyridine.[12] The first drug that was discovered and used in humans was nifedipine, as previously discussed. Love

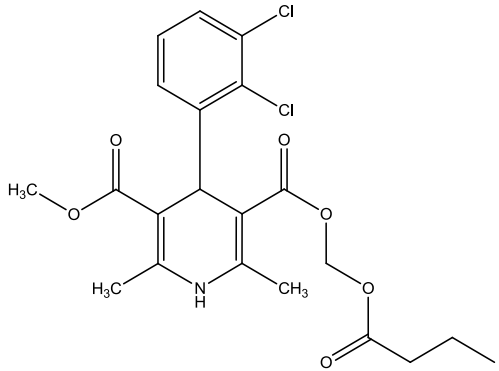
et al.[13] studied the molecular features that make this class of drugs active as CCB and explored which molecular changes could be made to improve their potency. He reported that in a series of substituted 2, 6-dimethyl-3, 5-dicarboethoxy-1, 4- dihydropyridines the sequence of activity increases with 4-substitution in the sequence H < Me < cycloalkyl < heterocyclic < phenyl and substituted phenyl. The presence of the 1, 4-dihydropyridine rings is essential, and N_1 in this ring has to be bound to hydrogen. Finally, the presence of an ester group at the C3 and C5 was found to be optimal. This finding was confirmed by several subsequent studies based on pharmacological and radioligand binding studies. The chemical structures for all the CCB drugs that were used in this analysis are shown in Figure 1.2.

Dihydropyridines

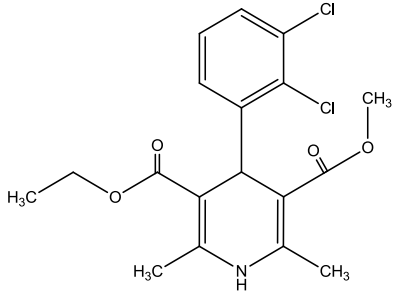
(a)
Amlodipine



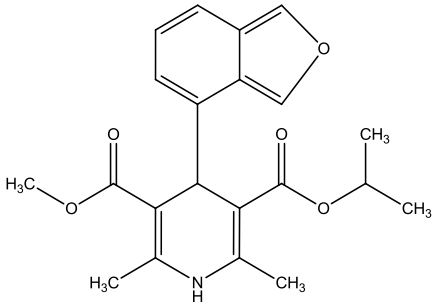
(b)
Clevidipine



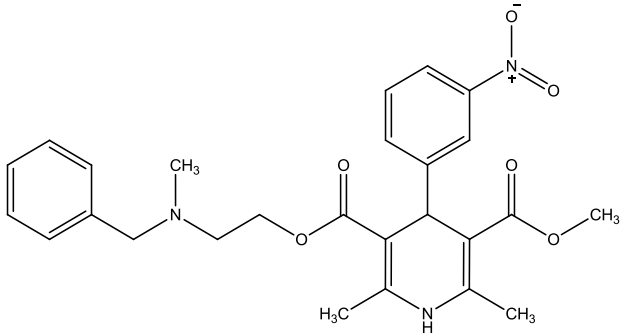
(c)
Felodipine



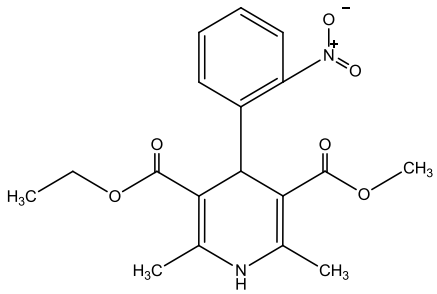
(d)
Isradipine



(e)
Nicardipine

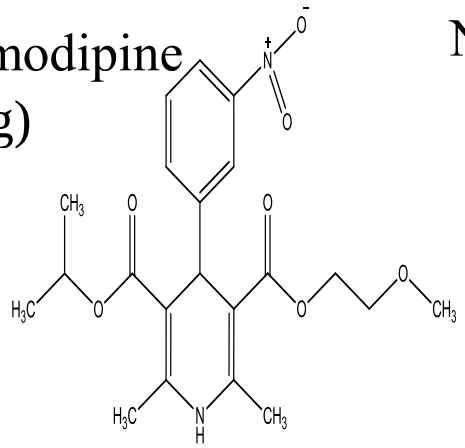


(f)
Nifedipine

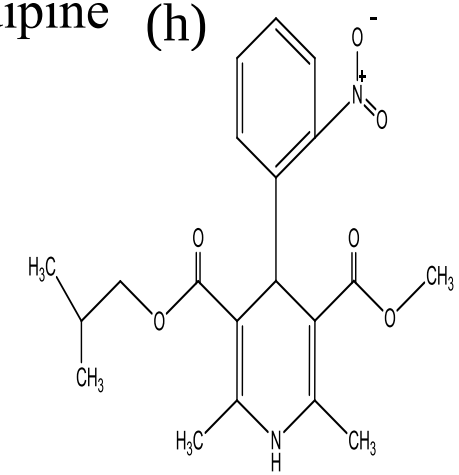


Dihydropyridines

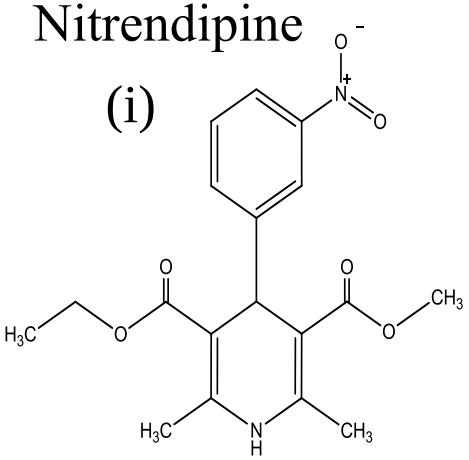
Nimodipine (g)



Nisoldipine (h)

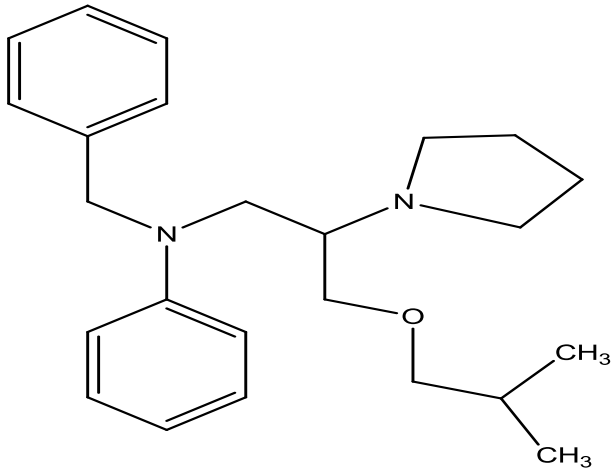


Nitrendipine (i)

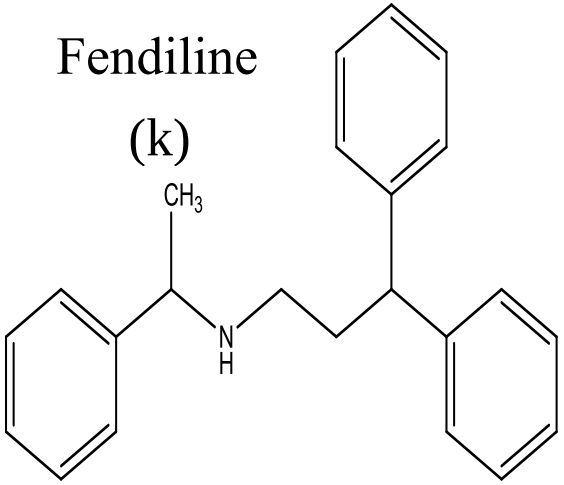


Phenylalkylamin

Bepridil (j)



Fendiline (k)



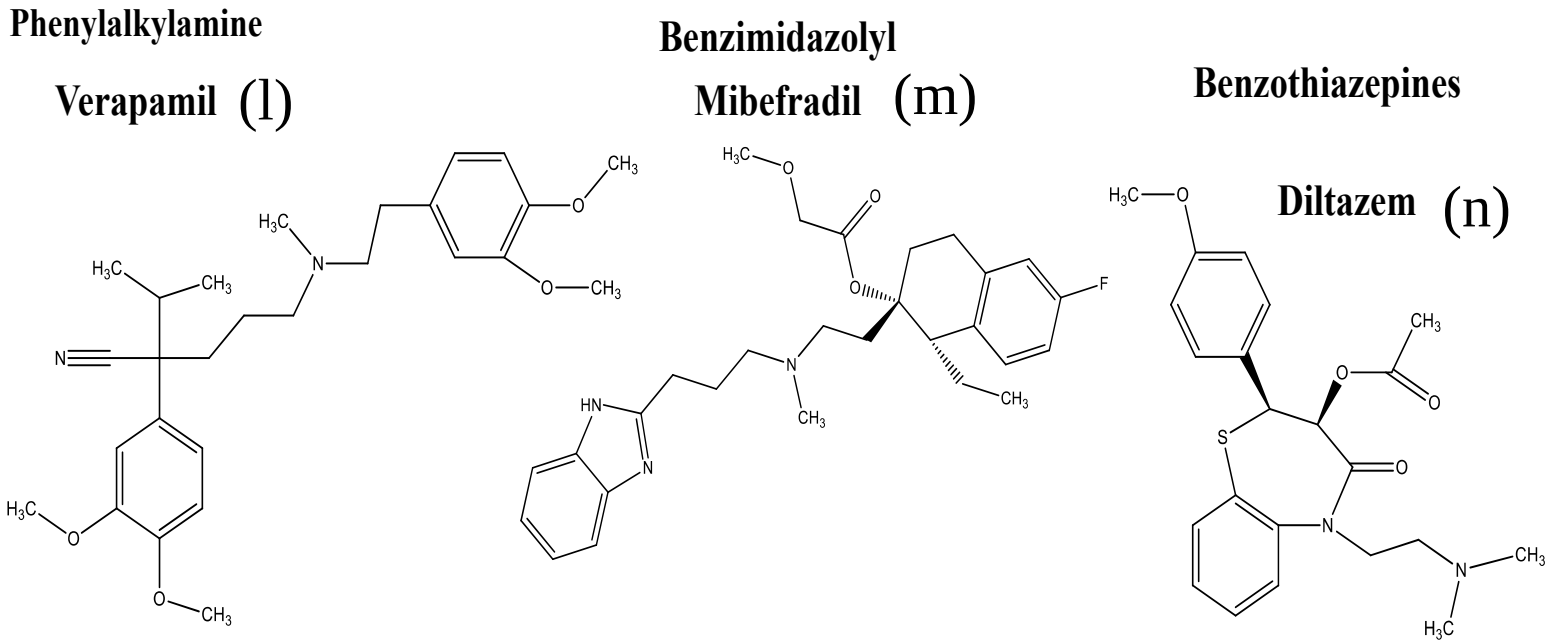


Figure 1.2 Chemical structures of classical CCB.

Dihydropyridines (a-i)
Phenylalkylamines (j-l), Benzimidazolyl (m) and Benzothiazepines (n).

Most CCB are administered as racemic mixtures. The *in-vivo* potency of each stereoisomer is different, because the metabolism of these drugs by cytochrome P-450(3A) is different for each isomer, resulting in stereo-selective drug metabolism and disposition. In most cases, one form is active and the other form is inactive or slightly active with respect to blockade of calcium channels. For example, for verapamil, the (-)-stereoisomer is more potent in the blockade of L-type voltage calcium channels in cardiac tissue and more potent as negative inotropic agent compared to the (+)-stereoisomer. The (-) isomer of verapamil is less available if the drug was administered by oral route because it has higher first-pass metabolism compared to the (+) isomer. However, there are two drugs (nifedipine and diltiazem) that do not have a chiral center; so, they are not administered as racemic mixtures.[14]

1.1.4 Quantitative-structure pharmacokinetic relationships

QSAR/QSPKR modeling is the process where molecular properties and descriptors of chemical structures are quantitatively correlated with biological activity or PK properties, such as clearance and Vd. QSAR simply asks: do compounds that share the same or similar biological activity have something similar about their chemical structures? Each model has underlying assumptions that primarily depend on the question that this model tries to answer. For example, when the goal of the model is to quantify structural similarity that imparts biological activity, the assumption is that other untested molecules with similar chemical features should produce similar activity.

A significant amount of information can be obtained from chemical structures that can be quantitatively correlated with biological activity, but which PC properties should be calculated and used to build a QSAR model? Based on the question that the model tries to answer, there are

different numbers of PC properties that should be used and there are several software packages that can help in obtaining these PC values. The PC properties to be measured are chosen after evaluation of a number of studies in the literature, and when strong evidence is found that these PC properties may affect the disposition of drugs. For example, Lipinski et al.[16] published a study after *in-vitro* evaluation of the solubility and permeability of 2200 compounds in which they had examined the structural properties. They came up with the "Rule of Five", or what is now known as the "Lipinski rules", after clear trends were observed between PC properties, solubility, and GI permeability of these compounds. This rule indicates that drugs that have molecular weight (MW) of more than 500 (D), logP value of more than 5, more than 10 hydrogen bound acceptors, and more than 5 hydrogen bound donors, are most likely to have poor oral absorption. The Lipinski rule is based on solid research and strong rationale, i.e., a higher number of hydrogen bonds increases the solubility of the drug in the gastrointestinal tract (GIT), making it more difficult for the compounds to break these bonds and penetrate into and through the lipid bilayer membrane of the GIT by passive diffusion.[15, 16] Veber et al.[17] conducted a study to examine the oral bioavailability in rate of 1100 drug candidates studied at GlaxoSmithKline. They found that the molecular flexibility, polar surface area (PSA), and hydrogen bond acceptors and donors (hydrogen bond counts) are important determinants of increasing oral bioavailability. They concluded that compounds are most likely to have good oral bioavailability when the number of rotatable bonds is $\leq$10, the value of PSA $\leq$140 $Å^2$ or the number of hydrogen bond acceptors and donors are $\leq$12.

Molecular properties are normally estimated using a variety of commercially available software, such as SYBYL, ACD lab, Dragon and HYBOT. Each software calculates several different 1D, 2D, 3D descriptors that may help to build a predictive QSAR model.[18, 19] Their

estimates actually depend on numerous factors, such as the number of compounds that were used in the original model validation, wide distribution between low and high-end values of the descriptors i.e., (MW, clogP, clogD, etc.) that were used, and sufficient coverage of chemical groups. Values of clogP or clogD are considered very important in QSAR and using incorrect values could lead to potential errors.[18, 20] Values of clogP and clogD are calculated by software because it is fast, cheap and highly accessible. Each software estimates these values based on experimental data of large number of databases, thus the clogP are most likely to be accurate for known structures. On the other hand, these *in-silico* estimates are not accurate like the one measured in the laboratory especially if it is for new compounds or derivatives.[20] Unfortunately, measurements of these properties (logD and logP) in laboratory are not always accessible, and the experimental techniques are very time consuming. There are many other difficulties like the presence of impurities with the compound or low solubility, or the need for co-solvents.[20]

A quantitative structure pharmacokinetic relationship (QSPKR) is a specific type of QSAR, which focuses only on the correlation between chemical structures and PK properties.[21] The underlying assumption of QSPKR is that variations in the PK properties of a series of compounds are dependent on differences and variations in their structural, physical, and/or chemical properties that are mainly obtained from chemical structures. QSPKR is an important approach in drug discovery and several models were developed to be used in this field.[21]

Badri[21] developed a QSPKR model for predicting human systemic PK properties of three pharmacological classes of drugs. These classes were opioids, β-adrenergic receptor ligands (β-ARL) and β-lactam antibiotics (β-LAs). Human and animal PK databases were used in this analysis to understand the relationship between molecular descriptors and PK behavior of the drugs. In this analysis, it was found that lipophilicity ($logD_{7.4}$) and molecular weight (MW) were

the most important molecular properties affecting the biologically relevant systemic PK properties. Furthermore, their QSPKR model were able to predict f_u, $Vdss^u$, Cl_{tot}^u and CL_{nonren}^u for molecules with $logD_{7.4}$ > -2.0 and MW < 350 D. On the other hand, there were some difficulties in predicting these values for compounds with $logD_{7.4}$ < -2.0 and MW > 350 D (e.g., β-LA) because most of these drugs require specific drug transporters for distribution and excretion.[21] The importance of this finding is that chemists know how to alter these molecular properties ($logD_{7.4}$ and MW) of compounds. Therefore, many new drugs could be discovered and/or existing drugs could be modified to develop improved drugs. In addition, the results were generalized across different classes of drugs that have dissimilar PC properties. Interestingly enough, it is not necessary to synthesize a molecule to predict PK properties, which is a major advantage in this method.[21]

Scientists are looking to increase vascular selectivity of CCB by different approaches to reduce the side effects of these drugs. However, it is very difficult, because the human body has various subtypes of Ca^{2+} channels with different tissue distribution. 1, 4-DHP is the largest group of CCB that have been developed in order to obtain higher tissue selectivity. The logical reason behind this superior vascular selectivity of DHP is that this group is bound preferably to inactivate voltage-gated calcium channels, which are available more in smooth muscle compared to cardiac muscle. In fact, cardiac muscle undergoes only brief periods of depolarization through the cardiac cycle, thus having brief inactivated state.[5, 22, 23] Several QSAR studies have been performed to enhance the intrinsic potency of dihydropyridine CCB and reduce the side effects by designing more potent and selective compounds.[22]

1.1.5 Pharmacokinetics (PK) of CCB

Even though the three prototypes of CCB are dissimilar in their chemical scaffold, they have similar PK features.[14, 24, 25, 26] Nifedipine, diltiazem and verapamil have a very good oral absorption, as they are almost completely absorbed following oral administration. However, the oral bioavailability of these drugs varies because they are subject to various degrees of presystemic metabolism by the liver and/or gut, which can lead into pronounced individual variation in systemic exposures.[21, 22, 23] The published oral bioavailability estimates of these drugs show that nifedipine has the greatest value (on average 50 to 60%) followed by diltiazem (on average 40%) and, finally, verapamil has the lowest value, around 20 to 30%.[24] Most CCB are metabolized in the liver predominately by cytochrome P-450(3A) to inactive or less active metabolites.[14, 23]

CCB have high plasma protein binding with diltiazem being the least plasma protein bound (80-85%). Renal clearance of the unchanged (parent) CCB is minimal.[13, 24] There is a high degree of variability in the PK data available about CCB drugs in the literature. For example, nifedipine showed high variability when PK data were compared between healthy volunteers from Germany and Japan after oral administration of 20 mg.[6] The same was found for healthy Caucasians and Mexicans after using a 10 mg capsule or a 20 mg slow release preparation of nifedipine.[6] However, there are several points that can be mentioned regarding CCB in general:
Most plasma half-lives of CCB are short, with some exceptions for the newer drugs of the 1,4 dihydropyridine type: amlodipine (which has the highest half-life), isradipine, nisoldipine and nitrendipine.[24] Oral bioavailability is increased by grapefruit juice for some of CCB which is mainly due to inhibition of CYP3A4 in the wall of small intestine.[23] The effect of grapefruit juice is most pronounced for drugs that have a high-first pass metabolism. Therefore, it may increase

the bioavailability of these drugs, which will then increase the exposure to the active drugs. In fact, the therapeutic index of CCB limits the clinical importance of the interaction with grapefruit, but for a few drugs (felodipine, nifedipine, verapamil, and nisoldipine) there is a higher interaction.[23] The volumes of distributions (Vd) of these drugs vary widely from 0.5 (clevidipine) to 20 L/kg (amlodipine). [13, 14, 24, 27] Clevidipine is a vasoselective, ultra short-acting, third generation CCB approved in 2008 by the FDA as an intravenous antihypertensive when oral therapy cannot be used. This drug is unlikely to be affected by renal or hepatic impairment because it is metabolized in the blood and tissue by esterases. Plasma protein bindings of the newer agents are very high, and are commonly in excess of 90%. For example, plasma protein binding of clevidipine is 99.5%. [27]

1.1.6 Pharmacodynamics (PD) of CCB

CCB interact with the L-type voltage-gated calcium channels in cell membranes. This action leads to different outcomes, which mainly depend on the site of action of these drugs. Thus, when these drugs affect the vascular smooth muscle, vasodilation will be seen. However, when these drugs affect cardiac muscle, contractility or conductivity of the heart will be decreased, and the automaticity in the pacemaker tissues in the heart will be affected.[5, 24 , 28] The dihydropyridine (DHP) group is more selective to vascular muscle, which leads to more vasodilatory effects, but only slightly affects AV and SA node conduction (negative inotropic effects).[5, 28] Due to the vasodilatory and hypotensive effects of these drugs, cardiac afterload usually decreases and heart rate increases to compensate for DHP-induced negative inotropic effects.[5, 28] Amlodipine and the newer agents in the DHP group are more vasoselective, with less negative inotropic effects.[26, 28] Nimodipine belongs to the dihydropyridine group, but it is

more selective for cerebral arteries than systemic arteries. On the other hand, the diphenylalkyamine group (verapamil) mainly affects cardiac muscle and thus shows larger negative chronotropic, dromotropic and inotropic effects.[14] Most of the side effects of this group are nonvascular in nature because they have little vasodilatory effects and thus cause less reflex tachycardia as compared with DHP. Diltiazem affects both vascular and nonvascular smooth muscle, demonstrating intermediate vasoselectivity.[15, 6, and 28]

2 RESEARCH HYPOTHESIS

To assess the relationships of molecular and physicochemical (PC) properties with human systemic PK properties for CCB and compare these relationships with other pharmacological classes of drugs.

2.1.1 Specific Aims

- To collect the biologically relevant molecular, PC and human systemic PK properties and evaluate their statistical distributions and analyze their collinearity.
- Evaluate the univariate relationships between PC and PK variables and compare these relationships across pharmacological classes of previously studied drugs.

3.1.1 Definition of biologically relevant PK variables

The PK variables that were used in this study include: 1) volume of distribution at steady state (Vd_{ss}); 2) total body clearance (CL_{tot}).

The term Vd_{ss} is used to quantify the distribution of the drugs in the body when every compartment in the body that has the drug is at equilibrium. [29] This volume of distribution is very important because it reflects only the distribution that is not influenced by elimination, thus it is a true primary, independent endpoint. However, it is affected by PPB of the drug.[29, 30] The term f_u indicates the fraction unbound of drug in plasma, thus it reflects the fraction of drug available for distribution, elimination and interaction with the target drug receptors. If f_u is low, distribution to the tissue may not be extensive and vice versa. In order to achieve an accurate estimation of Vd that takes into account the PPB of drugs, Vd has to be divided by f_u. Therefore, another term was used to correct for PPB, which is abbreviated as Vd_{ss}^{u} measures tissue distribution throughout the body in absence of PPB. [29, 30]

Another PK variable used is CL_{tot}, which is the most important PK property because it reflects the efficiency of all eliminating pathways in the body, such as the renal and metabolic pathways.[21, 29, 30] It is a very useful endpoint for the evaluation of elimination mechanisms, by elimination organs such as the kidney and liver. More specifically, renal clearance (CL_{ren}) represents the clearance of a parent drug and does not account for the metabolites' renal clearance.[29, 30]

CL_{ren} is a measure of the efficiency of the kidney in removing the drug from the body. Furthermore, the value of CL_{ren} can indicate the processes by which the kidney removes drugs (i.e., glomerular filtration, active tubular secretion, and tubular reabsorption). The term f_e is the

unchanged fraction excreted in urine after administration of the drugs by the IV route. The f_e values reported after IV administration of the drugs were used to calculate CL_{ren} from CL_{tot} as shown in Table 3.1. Oral f_e was not used in this analysis because it could be misleading if the parent compound is not fully absorbed.

Nonrenal clearance (CL_{nonren}) accounts for all elimination pathways except for CL_{ren} ($CL_{nonren}=CL_{tot}- CL_{ren}$).[29] The kidneys and liver are the most important organs for drug elimination because they receive large portions of cardiac output; therefore, they are the most thoroughly studied.[30] However, drug elimination through the kidneys is much easier to measure than liver clearance. Therefore, CL_{ren} is much easier to interpret. Since most of CCB are highly lipophilic compounds, it was assumed that CL_{nonren} is equivalent to hepatic metabolism. However, it may also be due to $CL_{extrahepatic}$ in blood (clevidipine) and other tissues, such as the lungs or brain. In most cases, only the unbound drug is available to the organ for the elimination, so it is important to correct for PPB. As a result CL_{tot}^{u}, CL_{ren}^{u}, and CL_{ren}^{u} were calculated by dividing by f_u. Table 3.1 shows the equations that were used to calculate the relevant biological PK properties.

Table 3.1. Estimation of *in-vivo* PK variables[29]

PK variables	Formula
Vd_{ss}	$Vd_{ss}= Vd_{cc}\left\{1+\frac{k_{12}}{k_{21}}\right\}$ or $Vd_{ss}=CL_{tot}\left[\frac{\frac{A}{\propto^2}+\frac{B}{\beta^2}}{\frac{A}{\propto}+\frac{B}{\beta}}\right]$ (Assuming two compartment body model)
Vd_{ss}	MRT_{sys} = $AUMC_{\infty}/AUC_{\infty}$ (after iv bolus) MRT_{sys} = $AUMC_{\infty}/AUC_{\infty}$ - Tinf/2 (after iv infusion) Vd_{ss} = CL_{tot}*MRT_{sys} (Using noncompartmental PK analysis)
Vd_{ss}^{u}	$Vd_{ss}^{u}=\frac{Vd_{ss}}{f_u}$
CL_{tot}^{u}	$CL_{tot}^{u}=\frac{CL_{tot}}{f_u}$
CL_{ren}	$f_e=\frac{U_{\infty}}{Dose}$, (U_{∞}) is the amount excreted unchanged in urine $CL_{ren}^{u}=CL_{tot}*f_e$(after IV administration)
CL_{ren}^{u}	$CL_{ren}^{u}=f_e*CL_{tot}^{u}$
CL_{nonren}	$CL_{nonren}=CL_{tot}-CL_{ren}$
CL_{nonren}^{u}	$CL_{nonren}^{u}=CL_{tot}^{u}-CL_{ren}^{u}$

3.1.2 Definition of PC Variables

The physicochemical properties (PC) that were used in this analysis are ionization constant in water (pK_a), molecular weight (MW), molar volume (MV), polar surface area (PSA), calculated logP (clogP), percent ionized and calculated clogD at pH 6.3 as well as at pH 7.4. The number of hydrogen bond acceptors (HBA), hydrogen bond donors (HBD), and rotatable bonds (nRot) were also used. HBA and HBD count the number of bonds between hydrogen (electropositive atom) and a strong electronegative atom that might receive a hydrogen bond, such as oxygen or nitrogen, respectively.[31, 32] The number of rotatable bonds is very important because it is a measure of molecular flexibility, which is defined as any single, non-ring bound on a nonterminal heavy atom (non-hydrogen). Molecular weight and molar volume are used to express the size of compounds, but MW is mostly used because it is easier to calculate. PSA is

the sum of the surfaces of polar atoms (usually oxygen and nitrogen) and attached hydrogen atoms.[32] The term pK_a is used to indicate the ionizability of a compound, which is a function of the basicity and/or acidity of the ionizable groups in the compound.[31, 32] In this analysis, the most dominant and basic pK_a was used. LogP is the $\log_{10}$ of the partition coefficient, which is used to indicate the affinity of a molecule or a moiety for a lipid phase (e.g., biological membranes); it is used to measure of lipophilicity of compounds.[31, 32] Most of the software that were used to predict logP have a large database of logP experimental data that help the software build relationships between the chemical structures and the values of logP, Then the software will predict the logP for the compounds based on the chemical structure of the molecule. In this analysis, clogP is predicted using ACD-solubility/DB 12.01 software. The obtained clogP values were compared with data obtained from another software, SciFinder Scholar, for the same drugs. There were very small differences between the values that were obtained. SciFinder Scholar software does not predict logP values; logP value will only appear if it was previously measured experimentally. LogP measures the partition coefficient of the molecule in its neutral form, and, if more accurate measurement of lipophilicity is required, $logD_{7.4}$ should be used. Since the ionization state depends on the pH of the environment and the pK_a of the molecule, lipophilicity and solubility will change as the pH changes.[32] Therefore, logP is not an appropriate measure of the molecule's lipophilicity in the physiological environment of the body. LogD is defined as the distribution coefficient between lipid and aqueous phases at different pH values; the most relevant pH values of the urine (6.3) and the pH of blood (7.4) to represent the relevant physiological pH. [31, 32]

3.2 Data Collection

3.2.1 Collection of PK variables

Systemic PK properties were taken from original PK research articles using the following specific criteria: Review articles were not used unless the original article was irretrievable. First, PK studies were identified when the drug was administered as an IV bolus in healthy volunteers. Using intravenous studies ensured that the effects of incomplete absorption or the effects of extensive first-pass metabolism did not confound the obtained PK values.

Healthy volunteers are preferable to avoid any concurrent disease or concomitant drugs that may affect the PK properties of drugs. Within each study, study design, dosing regimen, sampling schedule, assay procedures and PK analysis methods were evaluated. A study with a large number of volunteers is preferable. Generally, the two most important criteria used were the values of AUC_{extra} and LLOQ of the bioanalytical assay. In order to have confidence in the estimate for the terminal rate constant, a sampling schedule for at least 1-2 terminal half-lives should be used. When the contribution of the extrapolated area to the total area under the curve (AUC_{extra}/AUC_{∞}), is less than 20%, it is unlikely that there are problems with the sampling time schedule. The second criterion is to ensure that all measured plasma concentrations are above the lower limit of quantification (LLOQ) of the bioanalytical technique and that any concentration value below LLOQ should not be used. Experimental error will be high near the concentration of the LLOQ.[33] In the literature, many articles studied and evaluated the effects of CCB on hepatic and/or renal dysfunction population and, in such cases, data from the healthy control population was used instead. Also, many studies were focused on the effects of CCB with and without grapefruit juice. Under these circumstances, only the control group without the grapefruit was used in this analysis.

Body-weight corrected PK properties were calculated using the average body weight reported in the study; if the body weight was not reported, a 70 kg body weight was assumed. Vd_{ss} is the only volume that was used in this analysis; if it was not calculated or reported, two alternative approaches were used: For compartmental PK analysis, if the volume of the central compartment (Vd_{cc}) and micro-constants or macro-rate constants were calculated and reported in the study, Vd_{ss} was calculated using the equations in Table 3.1. The other approach was to use published mean or individual plasma concentration-time profiles, if they were provided, and to read the values electronically (GraphClick version 3.0.2).[33] Then the values of plasma concentration-time profiles were used to calculate the CL_{tot} and Vd_{ss} by non-compartmental analysis using (systemic) mean residence time (MRT_{sys}) as shown in Table 3.1. Using the latter approach is less accurate in the estimation of Vd_{ss}, as usually arithmetic means in the plasma concentration-time plot were provided or, in other cases single selected profile was provided preventing assessment of population variability. [28, 33] In general, the final selected studies were variable in terms of number of subjects, doses, sample collection times, techniques and methods of sample analysis and PK analysis methods (compartment and noncompartmental). Therefore, arithmetic means of the PK properties were calculated across studies for use in the final database used in this analysis. For most of the CCB, more than three studies were evaluated (see Appendix I).

Values for f_u in plasma was obtained from *in-vitro* PPB studies (measured mainly by equilibrium dialysis), after critical evaluation of these studies. The incubation time is expected to be sufficiently long to reach equilibrium, and f_u estimates were corrected for any volume shifts. Also, the experimental temperature, drug stability, drug concentration (therapeutic relevant concentration), drug stability and ligand stability, plasma source (fresh, frozen, individual donors),

and buffer (pH, concentration, composition) were reviewed to ensure that it imitates the physiological condition.[34]

To estimate the contribution of renal pathways to the overall elimination pathways, the fraction excreted unchanged in urine (f_e) was obtained (Table 3.1). The f_e was defined as accumulative amount excreted unchanged in urine after intravenous administration divided by the administered IV dose (bolus or infusion). .In the final analysis, f_e values were obtained from studies, where the authors collected the amount of parent drug that was recovered in the urine over a sufficient period of time (4-5 $t_{1/2}$). For several CCB (clevidipine, felodipine, nifedipine, nisoldipine, and nitrendipine), urinary excretion was assessed but no parent drug was quantified with the bioanalytical method employed. Therefore, f_e was set to zero, but CL_{ren} was not estimated due to imprecision, while CL_{nonren} was estimated as equal to CL_{tot}.

A summary of PK variables across studies (final values including ± SD for CL_{tot}, and Vd) is shown in **Appendix I,** and the final, individual PK studies are summarized in **Appendix II**. Comparisons between the CCB dose(s) that were used in this study and the therapeutic doses were conducted to ensure that each study used therapeutically relevant concentrations. Furthermore, the assumption of dose-proportionality or linear PK was verified using the AUC estimates at different doses of the CCB. This was done by collecting the values of AUC_∞ and c_{max} with each corresponding dose, for each drug from the PK studies that were used in this analysis. Then, AUC_∞ and/or c_{max} values were plotted as a function of the corresponding doses. These relationships were then evaluated to assess the linearity. A linear relationship was concluded if a straight line without an intercept was observed. CCB were further categorized using criteria discussed by Badri (Table 3.2).[21]

Table 3.2. PK classification of CCB

Classification	Criteria
Based on plasma protein binding (PPB)	
High PPB	$f_u < 20\%$
Intermediate PPB	$20\% < f_u < 80\%$
Low PPB	$f_u > 80\%$
Based on the renal clearance	
Net tubular reabsorption	$CL_{ren}^{u} < GFR$
Net glomerular filtration	$CL_{ren}^{u} = GFR$
Net tubular secretion	$CL_{ren}^{u} > GFR$
where GFR is the glomerular filtration rate (1.7 ml/min/kg)	

Table 3.3 Comparison of the therapeutic doses and the PK studies doses for different CCB

CCB	Doses in PK studies (µmol/kg)	Therapeutic Doses (µmol/kg)
Amlodipine	0.17-0.37	0.35
Bepridil	8.10-10.90	5.45
Clevidipine	0.001-0.44	0.06-1.00
Diltiazem	0.58-0.72	0.69-0.86
Felodipine	0.001-0.1	0.04-0.11
Fendiline	0.13	0.66-0.92
Isradipine	0.04-0.07	0.02
Mibefradil	0.50-2.30	1.00-2.50
Nicardipine	0.02-0.33	0.02-0.46
Nifedipine	0.04-0.17	0.21
Nimodipine	0.04-0.81	0.03-0.07
Nisoldipine	0.06-0.96	0.01-0.12
Nitrendipine	0.08-0.19	0.08-0.32
Verapamil	0.29-0.44	0.16-0.31

The therapeutic doses in this table were obtained from Micromedex® 2.0 Healthcare Series [intranet database]. Version 5.1. Greenwood Village, CO: Thomson Healthcare (2011).

3.2.2 Collection of PC variables

The physicochemical properties (PC) that were used in this analysis were pK_a, MW, MV, PSA, clogP, HBA, HBD, nRot, percent ionized and clogD at pH 6.3 as well as 7.4. These PC properties were chosen after evaluation of a number of studies in the literature providing strong evidence that these PC properties may affect the disposition of drugs. The physiochemical properties in this study were calculated using ACD-solubility/DB 12.01. The chemical structures were drawn for each drug using ChemDraw Ultra 12.0 suite and saved as a mol_2 file. Then, the saved file was moved to ACD-solubility/DB 12.01 to calculate the PC properties.

3.3 Data Analysis

3.3.1 Statistical distribution and collinearties

PK studies for the final fourteen CCB were collected from published literature; the drugs were classified according to their chemical structure into four groups; Dihydropyridine (9), phenylalkylamine (3), benzothiazepine (1), and benzimidazolyl (1).[9] Normal quantile plots were used to assess the normality of the mean. If the data for PK and PC were normally distributed, the mean and standard deviation were used to describe the central tendency and spread of the variable. PK variables (except f_u and f_e) were log-transformed due to wide dispersion and/or skewed distribution. JMP v.10 was used for all statistical computations.

3.3.2 Correlation analysis-PC variables

Correlation matrices between molecular descriptors were constructed to assess for collinearity. Throughout the study, if any two descriptors were found to be collinear ($r \geq 0.8$),

then one of them was removed from the univariate analysis based on the relative importance biological plausibility of these descriptors.

3.3.3 Correlation analysis-PK variables

Correlation matrices between PK variables were constructed to assess for collinearity. If any two variables were found to be collinear ($r \geq 0.8$), then one of them was removed from the univariate analysis based on the importance/biological plausibility of these variables.

3.3.4 Univariate relationships between PK and PC variables

Univariate linear regression of all PK variables versus each molecular descriptor was performed. The criteria that were chosen for a statistically significant relationship were both $r^2 \geq 0.3$ and $p < 0.05$. The slopes for all significant relationships were used to evaluate the direction and the magnitude of these relationships. These univariate relationships were then compared across other classes of drugs: Opioids, β-adrenergic ligands (β-ARLs) and benzodiazepines (BZ) that were previously studied.[18] JMP v10 was used for all statistical analyses.

4 RESULTS AND DISCUSSION

4.1.1 Comparison of PC properties

A total of 14 CCB were included in the final analyses, with 9 of these drugs are classified as dihydropyridine CCB. Most CCB drugs used clinically nowadays belong to this group. Therefore, CCBs were classified in some textbooks, as dihydropyridine and non-dihydropyridine CCB. Overall, CCB are mainly small molecules with molecular weights ranging from 300-500 Da, which indicates that these drugs are unlikely to have problems with their absorption (nonrestricted membrane diffusion) through the GIT membranes, as a consequence of their molecular weight.[4]

Table 4.1 shows the summary and the descriptive statistics of CCB PC properties, and Figure 4.1-4.8 shows the distribution of PC properties. Table 4.2 shows the statistical interpretation of CCB correlation matrices for the PC properties. Figure 4.8 (B) shows the correlation matrices of the CCB PC properties.

The nRot generally increases with molecular weight, and since molecular weights in this dataset are similar (1.6-fold difference across CCB), no major differences in the numbers of rotatable bonds of these drugs are expected.[17] HBA and PSA were found to be highly correlated with $r \geq 0.80$, and there is a logical explanation behind this correlation: Nitrogen and oxygen were used to measure these variables as explained above; therefore, it is expected to have a higher correlation between these two molecular descriptors. Thus, PSA was excluded from this analysis as a potential predictor variable. There were also two other molecular descriptors (molar volume and $logD_{6.3}$) that were excluded from the analysis as predictors because they were correlated with molecular weight and $logD_{7.4}$, respectively.

In general, CCBs are mainly basic drugs, which primarily have one pK_a that is related to

the basic group (-NH group). When the NH group is connected to the aliphatic part of the compounds the lone pair on the nitrogen atoms is available for reaction with the protons, and therefore the typical pK_a values for these compounds (amlodipine, diltiazem and verapamil) are between 8 to 9. However, when the NH group is connected to the aromatic ring (pyridine), only one electron from the nitrogen contributes to the aromatic ring. This leaves an unshared pair of electrons, which can accept a proton, and so this ring is measurably basic, and therefore the typical pK_a values for these compounds (nifedipine, nimodipine and nisoldipine) are between 2 and 3.[35] The pK_a value of the compounds relative to the urinary pH may be important in determining their renal tubular reabsorption.

Figure 4.1 indicates that MW has a small range and it is normally distributed. FRB has similar mean and median, but it seems to have a bimodal distribution. HBA is skewed to the left side, and HBD has a limited range with three outliers, amlodipine is on the high end, and on the lower end are verapamil and bepridil. LogP is normally distributed with a very large range (1000-fold range in partition coefficient, P). Also, $logD_{7.4}$ has a large range, and the mean value is close to the median value, but the distribution is skewed towards the left. Amlodipine was found to be an outlier in $logD_{7.4}$ (at the low end) and HBD (at the high end).

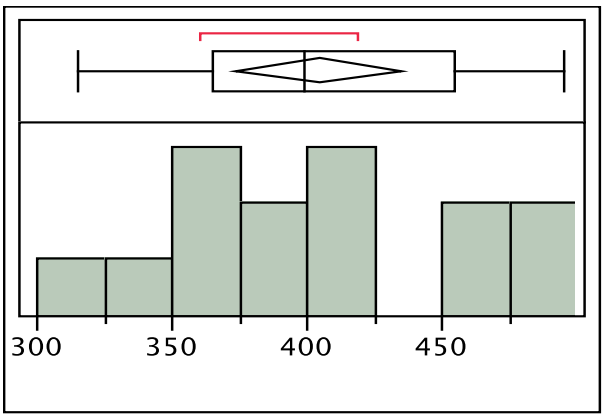


Figure 4.1 Distribution of MW (Da)

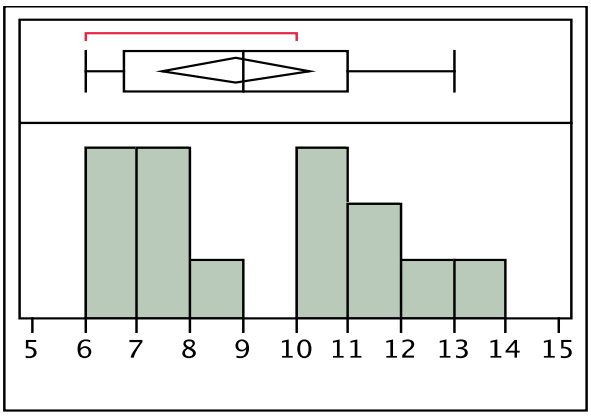


Figure 4.2 Distribution of nRot

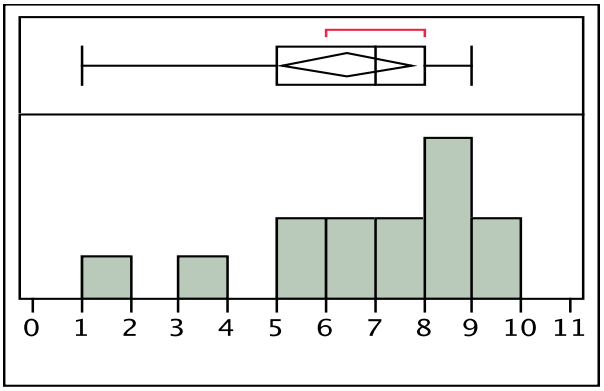


Figure 4.3 Distribution of HBA

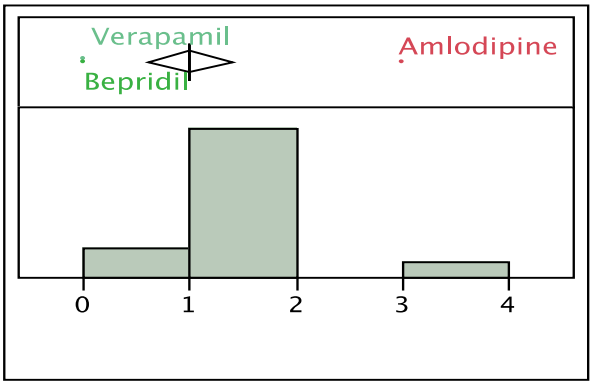


Figure 4.4 Distribution of HBD

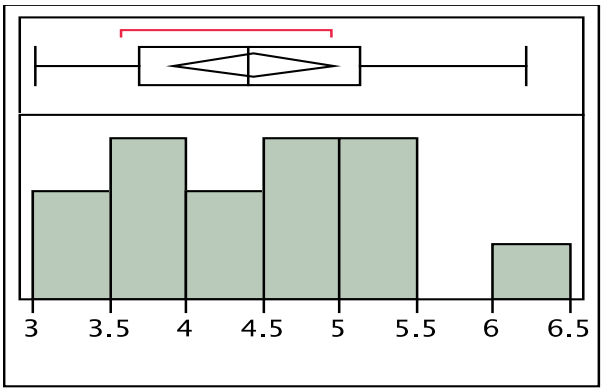


Figure 4.5 Distribution of logP

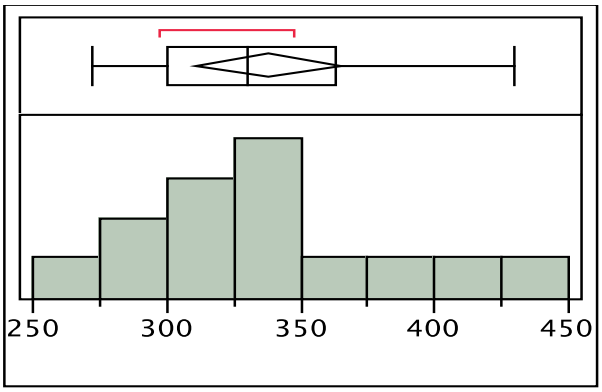


Figure 4.6 Distribution of molar volume (cm3/mol)

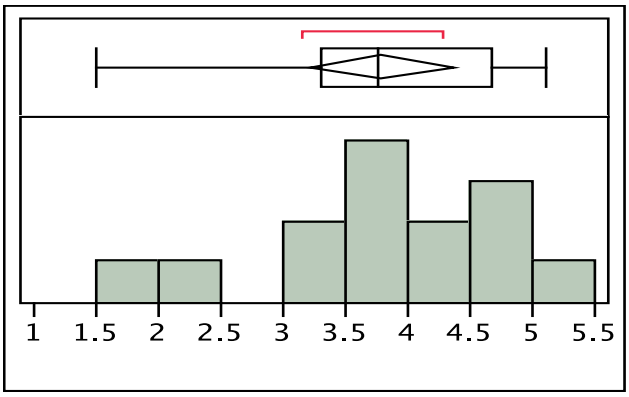


Figure 4.7 Distribution of $logD_{7.4}$

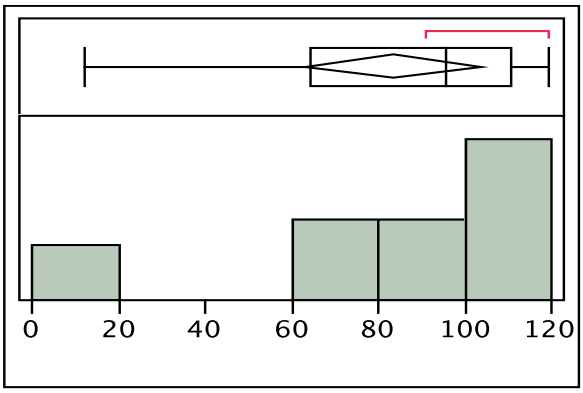


Figure 4.8 Distribution of PSA

Table 4.1 Summary of physicochemical properties of CCB

Drug	*MW*	*CCB classification*	*nRot*	*HBA*	*HBD*	*clogP*	*Molar Volume, (cm^3/mol)*	*pK_a*	*$logD_{6.3}$*	*$logD_{7.4}$*	*%Ionized @pH 6.3*	*%Ionized @pH 7.4*	*PSA (A^2)*
Amlodipine	409	Dihydropyridine	11	7	3	3.0	333	9.0	0.52	1.5	100	97	100
Bepridil	367	Phenylalkylamine	10	3	0	5.4	348	9.2	2.78	3.7	100	98	16
Clevidipine	456	Dihydropyridine	10	7	1	5.1	354	2.5	5.11	5.1	100	100	91
Diltiazem	415	Benzothiazepine	7	5	1	3.4	328	8.9	2.36	3.4	100	96	84
Felodipine	384	Dihydropyridine	6	5	1	4.8	301	2.7	4.76	4.8	100	100	65
Fendiline	315	Phenylalkylamine	7	1	1	5.2	306	9.5	2.36	3.2	100	99	12
Isradipine	371	Dihydropyridine	6	8	1	3.7	297	2.6	3.73	3.7	100	100	104
Mibefradil	496	Benzimidazolyl	12	6	1	6.2	418	11.9	3.32	4.3	100	99	67
Nicardipine	480	Dihydropyridine	11	9	1	4.9	390	7.3	3.85	4.6	91	45	114
Nifedipine	346	Dihydropyridine	6	8	1	3.6	272	2.7	3.58	3.6	100	100	111
Nimodipine	418	Dihydropyridine	10	9	1	4.1	345	2.8	4.05	4.1	100	100	120
Nisoldipine	388	Dihydropyridine	8	8	1	5.0	322	2.7	4.95	5.0	100	100	111
Nitrendipine	360	Dihydropyridine	7	8	1	3.8	289	2.8	3.81	3.8	100	100	111
Verapamil	455	Phenylalkylamine	13	6	0	4.0	429	9.0	1.49	2.5	100	97	64
25%*	**364**		***6.8***	***5***	***1***	***3.7***	***300***	***2.7***	***2.40***	***3.3***	***100***	***97***	*65*
Mean	***404.3***		***9***	***6***	***1***	***4.4***	***338***	***6.0***	***3.33***	***3.8***	***99***	***95***	***83***
75%*	***455***		***11***	***8***	***1***	***5.1***	***362***	***9.0***	***4.20***	***4.7***	***100***	***100***	*111*
SD	***52.5***		***2***	***2***	***1***	***0.9***	***47***	***3.5***	***1.32***	***1.0***	***2***	***15***	*35*
COV	***13%***		***27%***	***36%***	***68%***	***21%***	***14%***	***59%***	***40%***	***26%***	***2%***	***15%***	*42%*
Minimum	***315.5***		***6***	***1***	***0***	***3.0***	***272***	***2.5***	***0.52***	***1.5***	***91***	***45***	*12*
Maximum	***495.6***		***13***	***9***	***3***	***6.2***	***429***	***11.9***	***5.11***	***5.1***	***100***	***100***	*120*
Fold range	***1.6***		***2.2***	***9.0***	***4***	***2.1***	***1.6***	***4.9***	***9.80***	***3.4***	***1***	***2***	***10***

Drug	*MW*	*CCB classification*	*nRot*	*HBA*	*HBD*	*clogP*	*Molar Volume, (cm^3/mol)*	*pK_a*	*$logD_{6.3}$*	*$logD_{7.4}$*	*%Ionized @pH 6.3*	*%Ionized @pH 7.4*	*PSA (A^2)*
Difference	*180.2*		*7.0*	*8.0*	*3.0*	*3.2*	*157*	*9.5*	*4.59*	*3.6*	*9*	*55*	*108*
n	*14*		*14*	*14*	*14*	*14*	*14*	*14*	*14*	*14*	*14*	*14*	*14*

*25%: 25 percentile, *75%: 75 percentile.

Figure 4.8 (B) Correlation matrices of PC variables

Table 4.2 Statistical interpretations of PC variables correlation matrices

	MW	**nRot**	**HBA**	**HBD**	**clogP**	**MV**	**logD7.4**	**PSA**
MW	1.00							
nRot	0.76	1.00						
HBA	0.37	0.09	1.00					
HBD	-0.01	-0.05	0.25	1.00				
clogP	0.27	0.28	-0.36	-0.42	1.00			
MV	**0.85**	**0.92**	0.02	-0.27	0.42	1.00		
logD7.4	0.20	-0.21	0.24	-0.36	0.59	-0.03	1.00	
PSA	0.26	-0.07	**0.96**	0.39	-0.50	-0.15	0.19	1.00

4.1.2 Comparison of PK properties

Table 4.4 shows the final PK properties and their descriptive statistics for this dataset, and Figure 4.17(B) shows the correlation matrices of the CCB PK properties. Table 4.4 shows the statistical interpretation of CCB correlation matrices for the PK properties. Figures 4.9-4.16 show the distribution of various PK properties.

In the final dataset, there was a higher dispersion in the PK variables than the PC variables, which is clearly shown by a high standard deviation and the skewed distributions of these variables. Therefore, for the remainder of the analysis, the PK variables were log-transformed except for f_u.

The f_u values for most of the compounds were very low (less than 0.03%), and the f_u values were normally distributed. Two outliers (diltiazem and verapamil) had high f_u values.

In general, CCB exhibit somewhat similar PK properties, despite their differences in chemical structure and pharmacological activity: CCB are almost completely absorbed from the GIT, but they suffer from various degrees of first-pass metabolism, and, as a consequence, their oral bioavailability can be low, with wide inter and intra-patient variability (except amlodipine).

Mainly inactive metabolites are formed after hepatic metabolism, with the exception of clevidipine. Clevidipine was found to be an outlier in CL_{tot}, CL_{tot}^{u}, $CL_{nonren,}$ and CL_{nonren}^{u} due to the presence of an additional ester group on the molecule, as the compound is rapidly metabolized by esterases in blood and extravascular tissues into the corresponding carboxylic acid metabolite.[27] Renal elimination of the unchanged CCB is very low, thus CL_{ren} is a negligible route of CCB elimination. [29, 31, 32] Although there are studies in the literature that try to measure the parent drug concentrations in urine, for most of the compounds they were not able to detect it. Since most of these studies did not report the LLOQ, it was difficult to come up with estimates

of CL_{ren} values, and as a result only four values for CL_{ren} were estimated in this analysis.

The mean Vd_{ss} value in this dataset is 5.3 l/kg, which indicates that these drugs leave the vascular space and are highly distributed into body tissues. Amlodipine, with the highest value for Vd_{ss} of 20 l/kg, was found to be an outlier. After correction of PPB, bepridil was found to be an outlier with the highest value for Vd_{ss}^{u}.

The values for CL_{nonren} values in this dataset range between 7-131.5 ml/min/kg - with a mean value of 25.4 ml/min/kg, indicating that CCB have a moderate to high hepatic extraction ratio. Amlodipine is the only drug in this dataset that has a low extraction ratio. Most if not all, of CL_{nonren} is attributable to hepatic metabolism except for clevidipine (see above). As discussed before, hepatic metabolism results in the formation of inactive/less active metabolites primarily due to oxidation by cytochrome P-450(3A); ester hydrolysis (except for clevidipine) is thought to be a minor pathway of hepatic metabolism.[4, 14, 27]

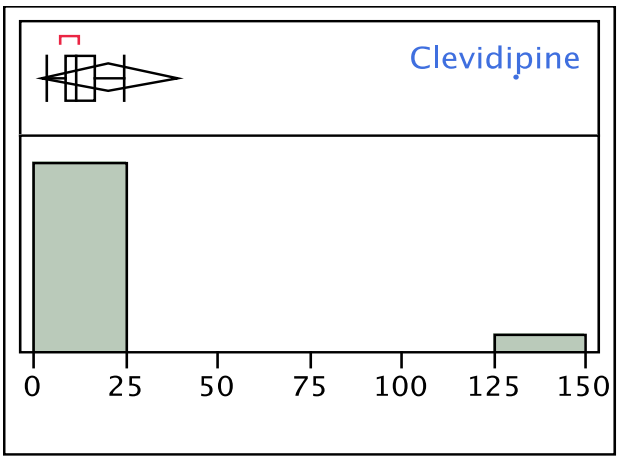


Figure 4.9 Distribution of CL_{tot} (n=14)

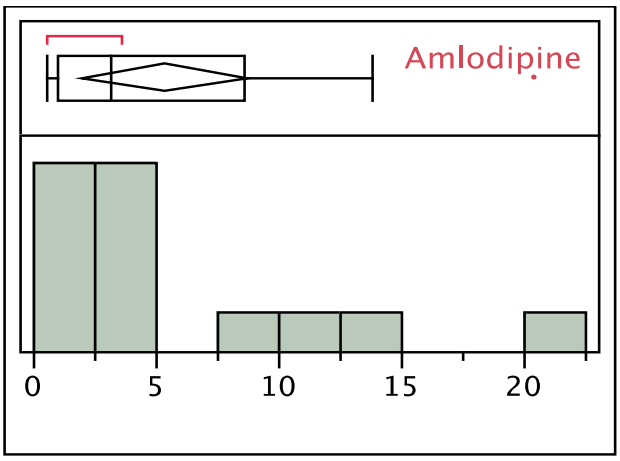


Figure 4.10 Distribution of Vd_{ss} (n=14)

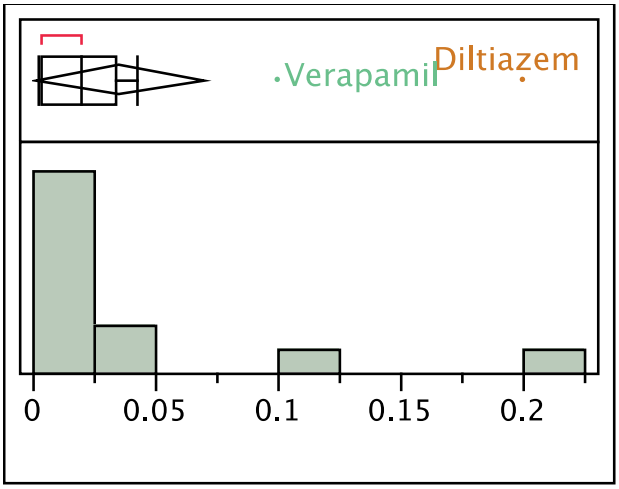


Figure 4.11 Distribution of f_u (n=13)

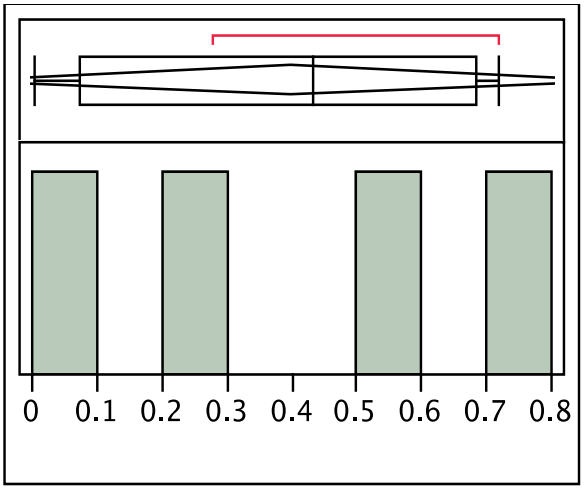


Figure 4.12 Distribution of CL_{ren} (n=4)

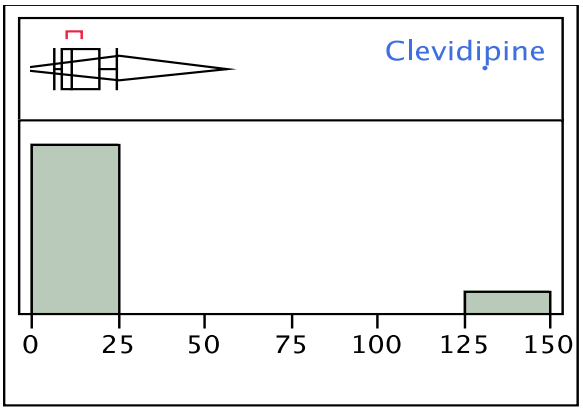


Figure 4.13 Distribution of CL_{nonren} (n=9)

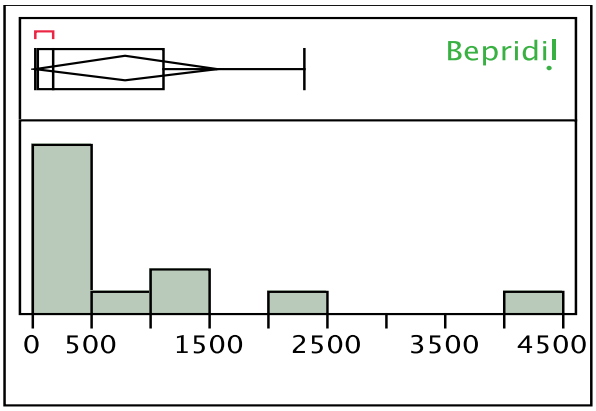


Figure 4.14 Distribution of Vd_{ss}^{u} (n=13)

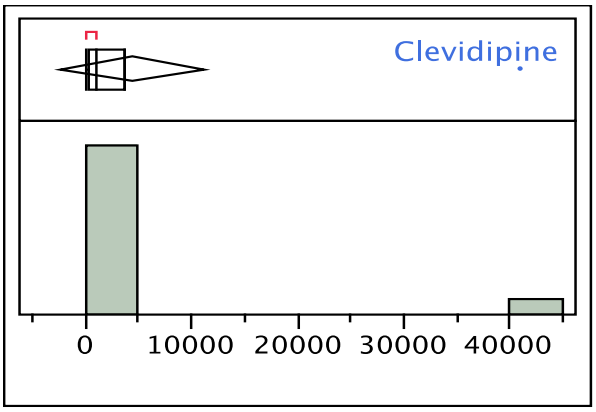


Figure 4.15 Distribution of CL_{tot}^{u} (n=13)

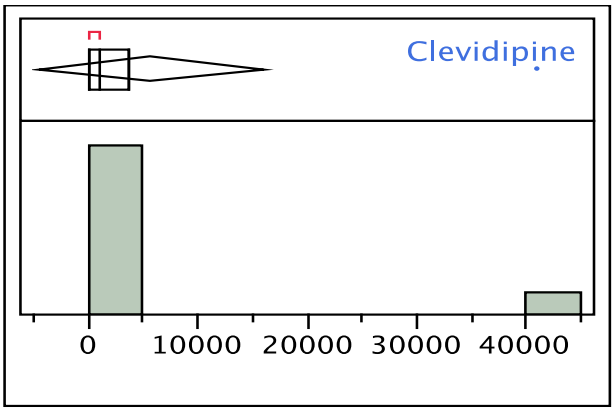


Figure 4.16 Distribution of CL_{nonren}^{u} (n=9)

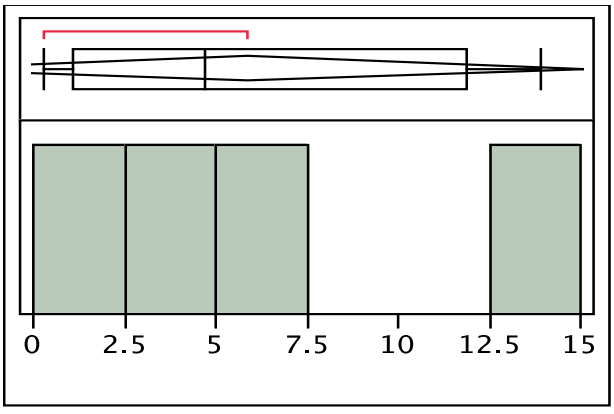


Figure 4.17 Distribution of CL_{ren}^{u} (n=4)

Table 4.3 Summary of the PK variables of CCB

Drug	CL_{tot} *[ml/min/kg]*	Vd_{ss} *[l/kg]*	f_e *[%]*	CL_{ren} *[ml/min/kg]*	CL_{nonren} *[ml/min/kg]*	f_u *[%]*	CL_{tot}^u *[ml/min/kg]*	CL_{nonren}^u *[ml/min/kg]*	CL_{ren}^u *[ml/min/kg]*	Vd_{ss}^u *[l/kg]*
Amlodipine	6.9	20.4	4.00%	0.278	6.7	2.0%	347	333	13.9	1022
Bepridil	8.7	10.1				0.2%	3783			4391
Clevidipine	131.5	0.57	0.00%		131.5	0.3%	41094	41093		178
Diltiazem	10.9	2.7	6.60%	0.717	10.2	20.0%	54	51	3.6	14
Felodipine	11.9	8.1	0.00%		11.9	0.4%	3389	3389		2300
Fendiline	17.4	13.8								
Isradipine	12.3	1.0				2.5%	492			42
Mibefradil	3.7	2.8				0.4%	925			700
Nicardipine	10.6	0.7	0.03%	0.003	10.6	1.1%	978	978	0.3	66
Nifedipine	7.4	0.7	0.00%		7.4	4.3%	175	175		16
Nimodipine	16.4	1.5				2.0%	822			74
Nisoldipine	11.3	3.6	0.00%		11.3	0.3%	3767	3767		1193
Nitrendipine	25.0	4.8	0.00%		25.0	2.2%	1125	1125		218
Verapamil	15.1	4.0	3.90%	0.587	14.5	10.0%	151	145	5.9	40

25%*	***8.4***	***1.0***	***0%***	***0.07***	***8.8***	***0.23%***	***261***	***160***	***1.1***	***41***
Mean	***20.6***	***5.3***	***1.6%***	***0.396***	***25.4***	***3.5%***	***4392***	***5673***	***5.9***	***789***
75%*	***16.7***	***11.8***	***3.95%***	***0.6***	***19.7***	***3.3%***	***3577***	***3578***	***11.8***	***1107***
SD	***32.3***	***5.9***	***2.5%***	***0.321***	***40.1***	***5.6%***	***10742***	***13356***	***5.8***	***1244***
COV	***157%***	***110%***	***157%***	***81%***	***158%***	***160%***	***245%***	***235%***	***98%***	***158%***
Minimum	***3.7***	***0.6***	***0.00%***	***0.003***	***6.7***	***0.2%***	***54***	***51***	***0.3***	***14***
Maximum	***131.5***	***20.4***	***6.6%***	***0.717***	***131.5***	***20.0%***	***41094***	***41093***	***13.9***	***4391***
Fold range	***35.5***	***35.8***		***251.6***	***19.7***	***100.0***	***756.1***	***809.5***	***52.6***	***324.1***
Difference	***127.8***	***19.9***	***6.60***	***0.715***	***124.8***	***19.8%***	***41039***	***41042***	***13.6***	***4378***
n	***14***	***14***	***9***	***4***	***9***	***13***	***13***	***9***	***4***	***13***

*25%: 25 percentile, *75%: 75 percentile.

Figure 4.17 (B) Correlation matrices of PK variables

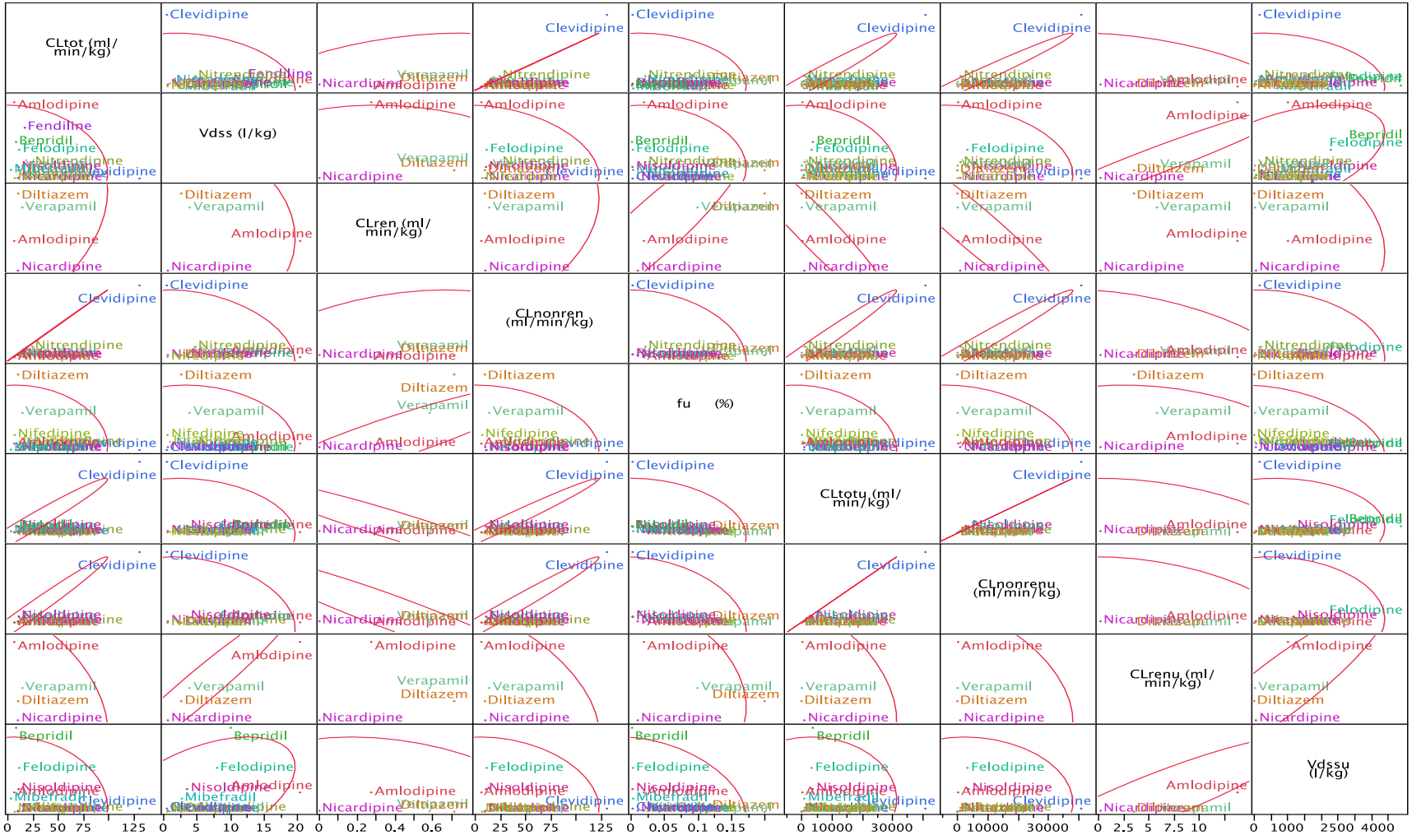


Table 4.4 Statistical interpretation of CCB PK variables correlation matrices

	CL_{tot}	Vd_{ss}	CL_{ren}	CL_{nonren}	f_u	CL_{tot}^{u}	CL_{nonren}^{u}	CL_{ren}^{u}	Vd_{ss}^{u}
CL_{tot}	1.00								
Vd_{ss}	-0.24	1.00							
CL_{ren}	0.43	-0.12	1.00						
CL_{nonren}	**1.00**	-0.30	0.35	1.00					
f_u	-0.16	-0.14	**0.91**	-0.25	1.00				
CL_{tot}^{u}	**0.98**	-0.20	**-0.95**	**0.99**	-0.23	1.00			
CL_{nonren}^{u}	**0.99**	-0.27	**-0.95**	**0.99**	-0.29	**1.00**	1.00		
CL_{ren}^{u}	-0.51	**0.97**	0.09	-0.54	-0.24	-0.40	-0.41	1.00	
Vd_{ss}^{u}	-0.18	0.51	-0.29	-0.20	-0.35	-0.04	-0.10	**0.91**	1.00

CCBs varied in their total body clearance from 3.7 (mibefradil) to 131.5 ml/min/kg (clevidipine). The clevidipine clearance value is an outlier within this dataset, as it is the highest value exceeding liver blood flow (LBF) and cardiac output due to extrahepatic tissue and blood hydrolysis (see above).[27] As a result, clevidipine has the shortest $t_{1/2,}$ which makes it unique among the other DHP CCB. Clevidipine also has a greater vasoselectivity with an ultra-short half-life that is required when rapid and controlled reduction of blood pressure is necessary.[27] Clevidipine is used as an intravenous antihypertensive agent because it is easily titratable with a low risk for toxicity and drug-drug interactions.[27]

Overall, CCB were found to be highly metabolized, and their CL_{nonren} values approached or exceeded LBF (20 ml/min/kg), indicating that they have a moderate to high hepatic extraction ratio (ER_{hep}), except for amlodipine. Since CCB are highly lipophilic drugs, CL_{nonren} in this dataset was assumed to be due to hepatic elimination, which means that CL_{nonren} represents CL_{hep}, except for clevidipine.

Smith et al.[40] studied the *in-vitro* hepatic metabolism of dihydropyridine CCB and found that these compounds are essentially converted to the pyridine metabolites (human and animals) as the common, initial clearance step. He reported the intrinsic clearance (CL_{int} in ml/min/kg) of 6 dihydropyridine CCB, namely amlodipine (11), nitrendipine (88), felodipine (75), nicardipine (131), nisoldipine (190), and nilvadipine (100). The intrinsic clearance is defined as the intrinsic ability of liver to remove a drug in the liver in absence of flow limitations. These values clearly indicate that the liver efficiently clears these drugs, but because they are highly plasma protein bound (binding-restricted drugs), only moderate ER_{hep} can be seen for some of these drugs. The CL_{nonren}^{u} values

calculated using the current dataset of CCB were much higher than the previously reported estimates of CL_{int} (see table 4.5). After correction for PPB, it was observed that the values of CL_{nonren}^{u} exceeded the liver blood flow for all the drugs in this dataset. However, when Smith et al[40] estimated CL_{int} based on studies which were conducted in different animals species (rat, dog and monkey) from literature and extrapolated to human CL_{int}. However, the model used for the estimation was not described in the study. Furthermore, the methods of estimation of CL_{int}, and the assumptions underlying each of studies - Smith et al.[40] and this study - could be potential explanations for the discrepancies in the estimates of CL_{int} values of CCB from these studies. An additional explanation would be that each study used different PPB estimates; since it is a very small value, the final results for CL_{int} could change significantly. However, the values from both studies clearly indicate that these CCB are highly metabolized.

Table 4.5. Comparison between CL_{int} from Smith et al.[40] and CL_{non}^{u} from the current study

CCB	CL_{int} (ml/min/kg)[40]	CL_{nonren}^{u} (ml/min/kg)
Amlodipine	11	333
Felodipine	75	3388
Nicardipine	131	977
Nisoldipine	190	3766
Nitrendipine	88	1124

Waterbeemed et al.[35], studied the correlation between the CL_{int} as a function of $logD_{7.4}$ for nine CCB, but did not perform any regression analysis on this relationship (CL_{int} was obtained by *in-vitro-in-vivo-extrapolation*). These published data were read electronically (GraphClick version 3.0.2), and linear regression was performed on the log-transformed values of CL_{int} as function of log $D_{7.4}$ to obtain the slope (S = 0.58) and

regression coefficient (r^2 = 0.77, n=9) shown in Figure 4.17C. Results obtained from the current QSPKR study (n = 9, S = 0.56, r^2 = 0.42) indicated similar direction and magnitude of the trend (slope). This suggests that the effect of lipophilicity on hepatic/nonrenal clearance of CCB is very consistent across both studies.

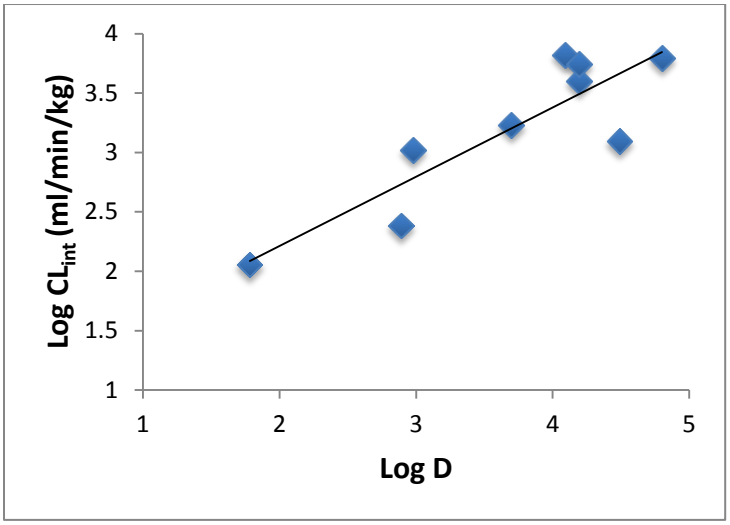


Figure 4.17(C) Univariate relationship of log-transformed CL_{int} as a function of $logD_{7.4}$ using data from Waterbeemed et al.[35],

Renal clearance typically decreases with increased in lipophilicity because compounds that have a higher lipophilicity, like CCB, should have a higher passive permeability (tubular reabsorption).[36] Another reason why renal clearance is low for these drugs is the high PPB (>80%), which means that these drugs will be less available for glomerular filtration.[36] The contribution of CL_{ren} to CL_{tot} is negligible because the renal clearance of these drugs is less important compared to CL_{nonren}, and because it is difficult to find studies that allow valid estimates on CL_{ren} of CCB after IV administration. In fact, when these studies were found, most of them did not have detailed information. For

example, Raemsch et al.[37] studied the metabolism and excretion of intravenous nimodipine and mentioned that "no unchanged drug was found in the urine during the first 24 hours (>10 $t_{1/2}$)", but the lower limit of quantification (LLOQ) of this study was not mentioned. Under these circumstances, it could be that there is no parent drug excreted in the urine, or there is a small percent of the parent drug excreted in the urine and the bioanalytical technique is not able to detect it. The only way to differentiate between the two cases is by knowing the LLOQ of the assay that was used. For this reason, nine values of CL_{ren} are missing in this analysis. However, the value of CL_{ren} is very small compared to the value of CL_{nonren}, so it possible to use these studies to estimates the CL_{nonren}.

CCB varied in their CL_{ren}^{u} values from 0.3 (nicardipine) to 13.9 ml/min/kg (amlodipine), which in the case of nicardipine CL_{ren}^{u} is less than glomerular filtration rate (GFR), indicating that it undergoes net tubular reabsorption. However, on the other hand, amlodipine exceeds GFR, indicating that it undergoes net tubular secretion.[41]

The volume of distribution of these drugs is high; most of the values (approximately 85%) exceed the total body water. Clevidipine shows the lowest Vd_{ss} (0.6 l/kg), while amlodipine shows the highest Vd_{ss} (20.4 L/kg). This would indicate that most of these compounds are extensively distributed into body tissues. The distribution of the drugs in the body is mainly dependent on the lipophilicity, which is expressed by $logD_{7.4}$, and PPB. In fact, PPB for most of CCB is very high, exceeding 90% except for diltiazem (80%). Therefore, free (unbound) drugs that are available to produce the pharmacological action are very limited.

Furthermore, PPB is also important in terms of drug disposition, which influences

the Vd_{ss} and the total clearance of these drugs. After correction of PPB, it was observed that the Vd_{ss}^{u} exceeds total body water volume or total body weight, indicating extensive distribution in to the body tissues.

CL_{ren} values for CCB are below GFR, but would have to be corrected for PPB; CL_{ren}^{u} values become higher than GFR for amlodipine, diltiazem and verapamil, which suggest net tubular secretion. The theoretical concepts of the relationships between PPB and renal clearance of drugs are well defined and are consistent with our finding between renal clearance and f_u.[36] As f_u increased, renal clearance increased (r^2=0.91). There was a higher correlation between CL_{tot} and CL_{nonren} (r^2=1.0), which suggests that the non-renal pathway is mainly responsible for the total clearance of these drugs and the renal clearance pathway is very small.

4.1.3 QSPKR

The results of the univariate regression between PK and PC for the complete dataset, with and without amlodipine, are shown in Table 4.6 and Table 4.7, respectively. Amlodipine was separated out because it was an outlier in one of the PC and one of the PK properties, and because it had different PK properties compared to the other CCB.

The question remains of why amlodipine is different compared to other CCB. Amlodipine has the largest plasma half-life across all CCB, and good oral bioavailability (60 to 65%) because it has lower presystemic or first-pass metabolism.[41] Oral CCB have good absorption characteristics, as discussed before, but metabolic enzymes inactivate a large fraction of these drugs and reduce their oral bioavailability. Therefore, the CCB that is subjected to first-pass metabolism requires a large dose to be administered to ensure

that an effective concentration of active drug exits the liver and reaches the systemic circulation; otherwise, it will not be available in the site of action. Clinically, amlodipine is administered in a smaller dose than other CCB because it is not subjected to a large first-pass metabolism.[42, 43] Amlodipine is a basic drug with a pK_a of 8.97, which means that, at physiological pH, it is mainly ionized (positively charged). This positive charge of the amino group (pK_a is 8.7) allows this drug to bind with a high affinity to cell membranes in peripheral body tissue that are mainly negatively charged.[42] Therfore, despite its large PPB, amlodipine has the largest Vd_{ss} (21 L/kg) across all CCB.[42, 43] Amlodipine has a PK profile that is unlike any of the other CCB available in the market or present in this study. It. For all of the above reasons, the analysis was performed with and without amlodipine.

In this analysis, there was no single PC variable showing a high relationship such that a single PC property could be used alone to predict a human PK property; however, there were trends, suggesting a significant contribution of some PC properties to certain human PK properties. After critical evaluation of several published studies, only particular PC properties were prespecified earlier in this analysis.[15, 16, 17] These PC properties that found to affect the biologically relevant, systemic PK properties were chosen. PC properties that affect the drugs after giving by oral route were not used in this analysis.

Figures 4.18-4.26 shows the univariate relationships between PC vs. $logD_{7.4}$ for the complete set and **Figures** 4.27-4.35 for the reduced set without amlodipine.There is a significant negative relationship between lipophilicity measured by $logD_{7.4}$ and f_u (Figure 4.23). This finding is consistent with other classes of drugs such as opioids,

benzodiazepines, β-blockers and anti-arrhythmic drugs in humans.[21, 44,45] Waterbeemd et al.[35] studied this relationship between plasma protein binding and lipophilicity ($logD_{7.4}$) of 150 acidic, basic, and neutral compounds. He found a sigmoidal relation between f_u and $logD_{7.4}$ and this relationship became linear after transformation of the data to a logarithmic form. He also observed that basic compounds that have $logD_{7.4} > 2$ will have lower f_u. Obach et al.[14] also studied the same relationships with a larger number of compounds (554) and found a trend in the data in that drugs with a higher $logD_{7.4}$ had a higher PPB. Basic compounds like CCB are expected to bind to α_1-acid glycoprotein due to an electrostatic interaction with acidic residues.[30]

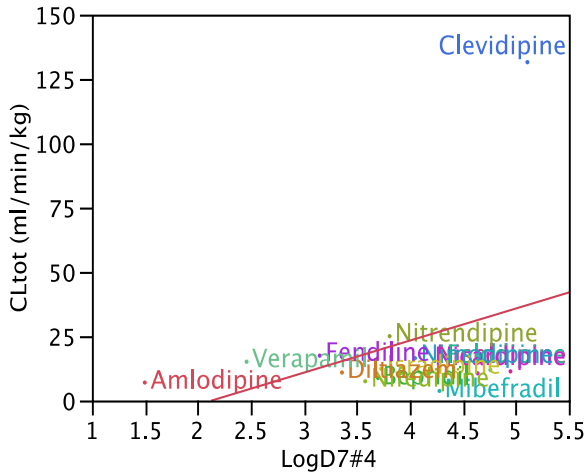


Figure 4.18 CL_{tot} vs. log $D_{7.4}$

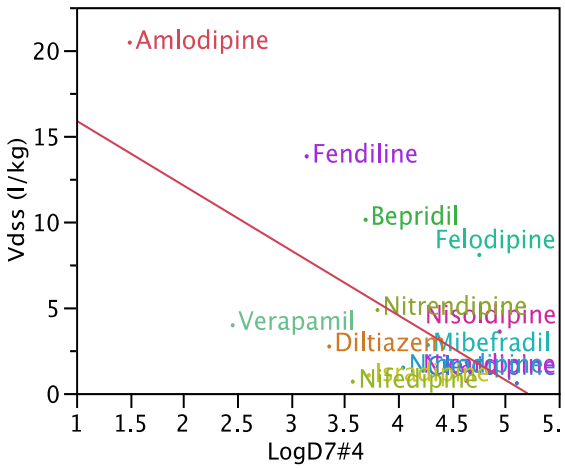


Figure 4.19 Vd_{ss} vs. log $D_{7.4}$

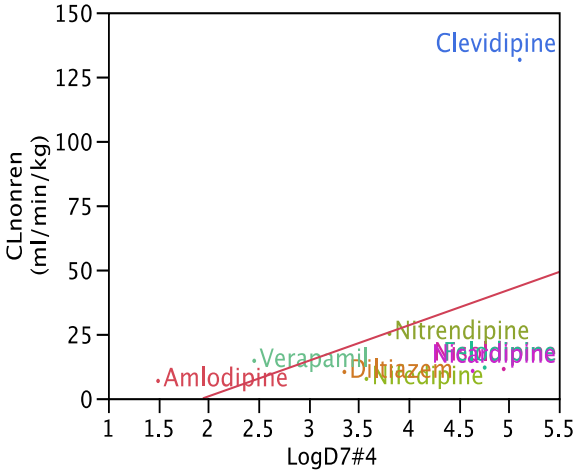


Figure 4.20 CL_{nonren} vs. log $D_{7.4}$

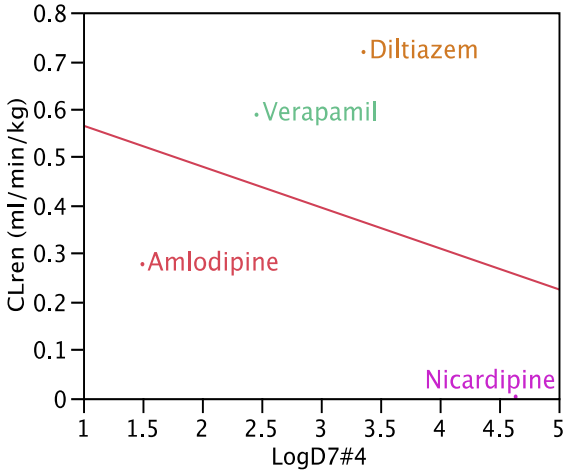


Figure 4.21 CL_{ren} vs. log $D_{7.4}$

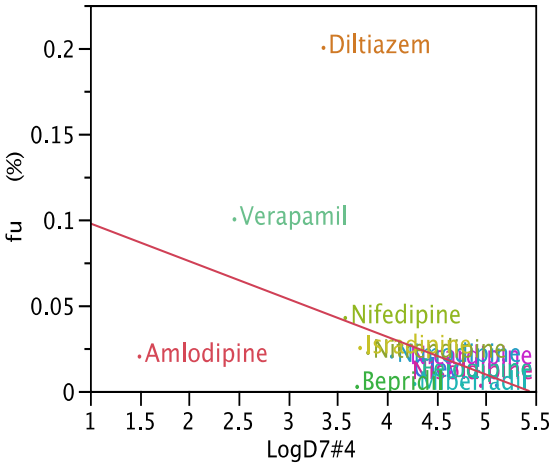


Figure 4.22 f_u vs. log $D_{7.4}$

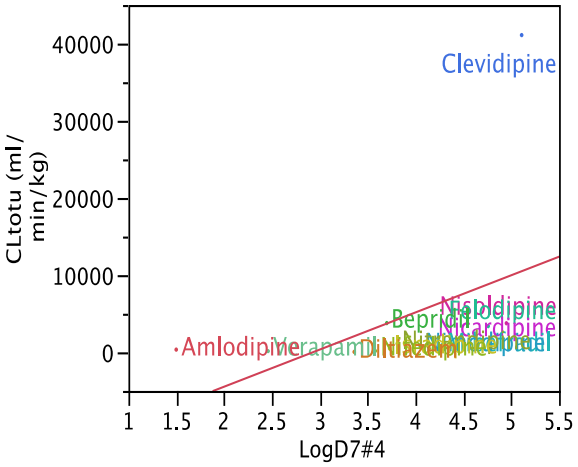


Figure 4.23 CL_{tot}^{u} vs. log $D_{7.4}$

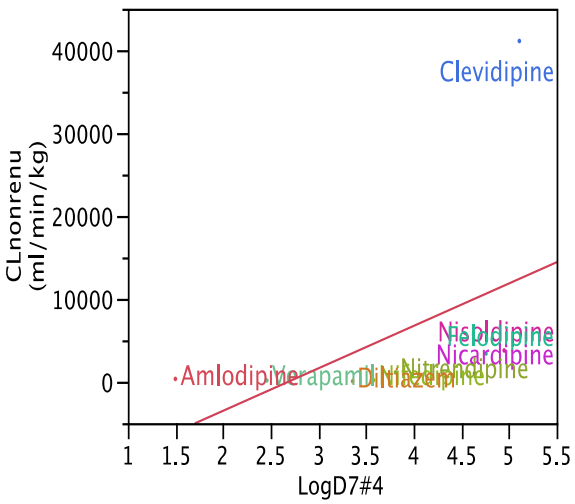


Figure 4.24 $CL_{non\ m}{}^{u}$ vs. $logD_{7.4}$

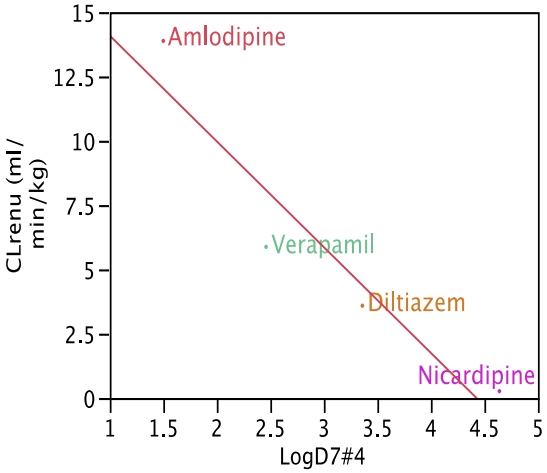


Figure 4.25 $CL_{ren}{}^{u}$ vs. $logD_{7.4}$

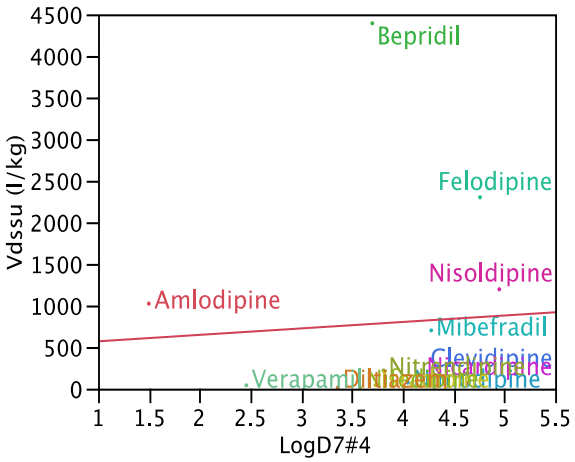


Figure 4.26 $Vd_{ss}{}^{u}$ vs. $logD_{7.4}$

There were no significant relationships between Vd_{ss}, $Vd_{ss}{}^{u}$ and $logD_{7.4}$, which is inconsistent with several references.[15, 17] Waterbeemd et al.[35] studied the relationship between the unbound volume of distribution and $logD_{7.4}$ and found a strong relationship. To understand more about this relationship, we have to identify other factors that can affect the volume of distribution. A major factor that affects the Vd_{ss} is the affinity for the cell membranes in peripheral tissues. The two physiochemical properties that impact this affinity are the compound's basicity and its intrinsic lipophilicity. [15, 17] In the case of

CCB, they are basic drugs fully ionized at physiological pH; thus, there is an ion-pair interaction between the charged acidic groups of the membranes and the basic center of the drug. To separate the effects of ionization from the intrinsic lipophilicity, pK_a values were divided in this dataset and used the part of the data that have similar pK_a values. This relationship between $logD_{7.4}$ on the drugs that has similar pK_a (7.3-9.8) and Vd_{ss} was studied. It was found that r^2 increased from 0.08 to 0.53 (p-value= 0.06, n=6). Although this relationship is still not statistically significant and has a small sample size, an improvement in this relationship with the expected trend was seen. It may have required a larger sample size to show a statistically significant relationship especially for basic drugs that are fully ionized at physiological pH. On the other hand, a significant relationship between logP and Vd_{ss}^{u} was found (r^2=0.54, n=12); logP depends on the partitioning of the neutral molecules between the aqueous phase and the organic phase.[47]

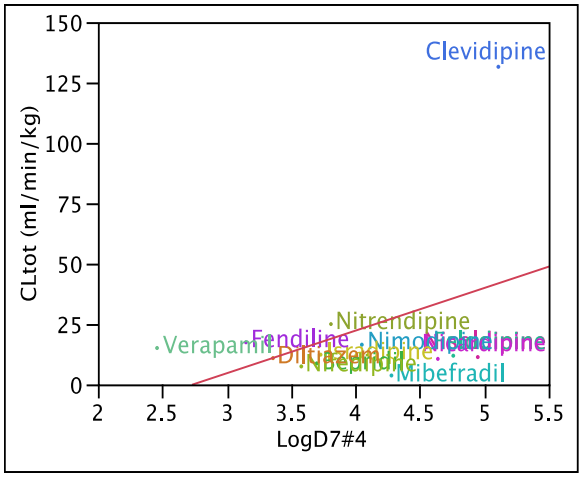


Figure 4.27 CL_{tot} vs. $logD_{7.4}$ (without amlodipine)

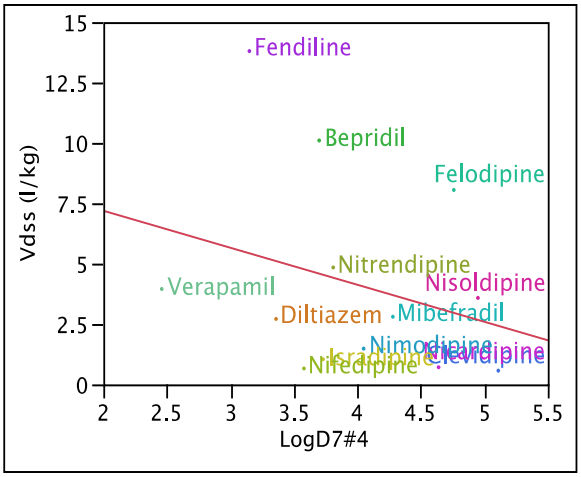


Figure 4.28 Vd_{ss} vs. $logD_{7.4}$ (without amlodipine)

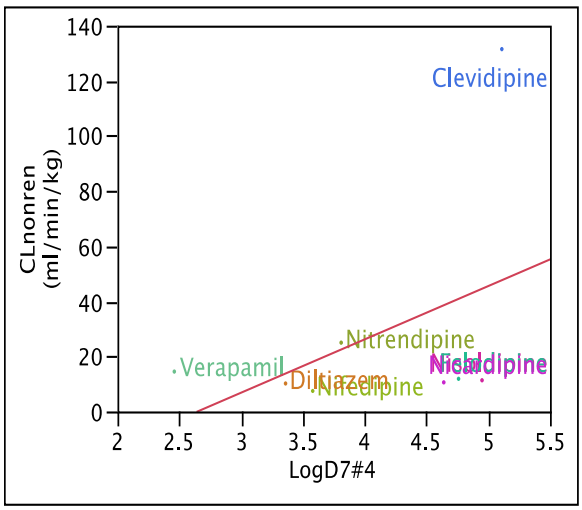


Figure 4.29 CL_{nonren} vs. $logD_{7.4}$(without amlodipine)

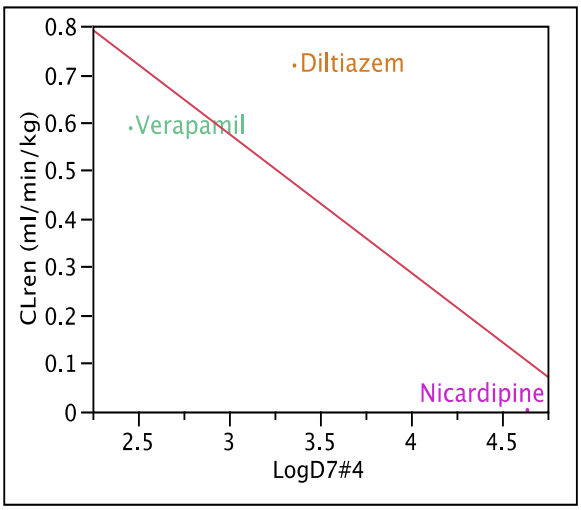


Figure 4.30 CL_{ren} vs. $logD_{7.4}$(without amlodipine)

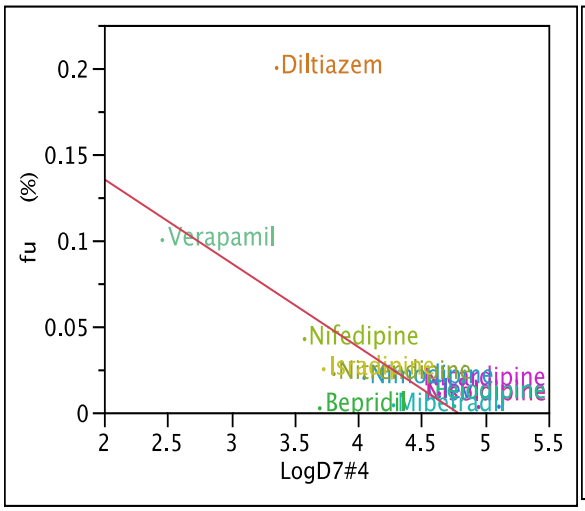


Figure 4.31 f_u vs. $logD_{7.4}$(without amlodipine)

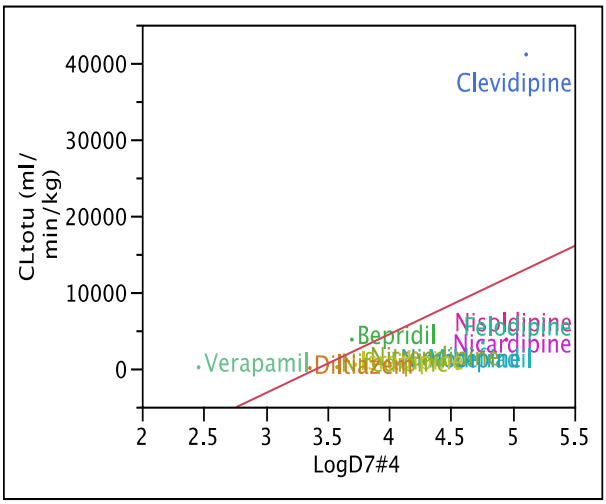


Figure 4.32 CL_{tot}^{u} vs. $logD_{7.4}$(without amlodipine)

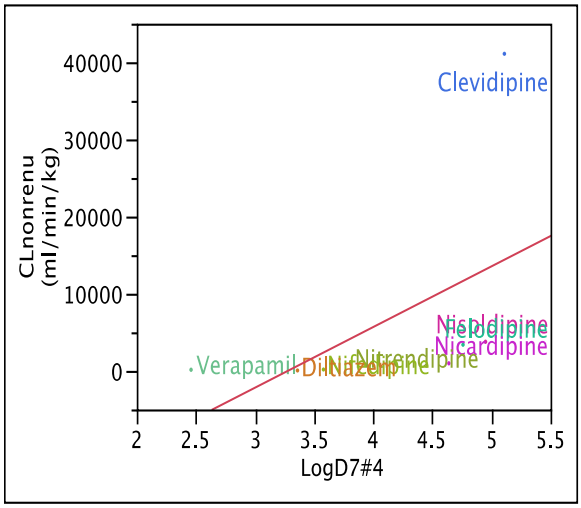


Figure 4.33 CL_{nonren}^{u} vs. $logD_{7.4}$ (without amlodipine)

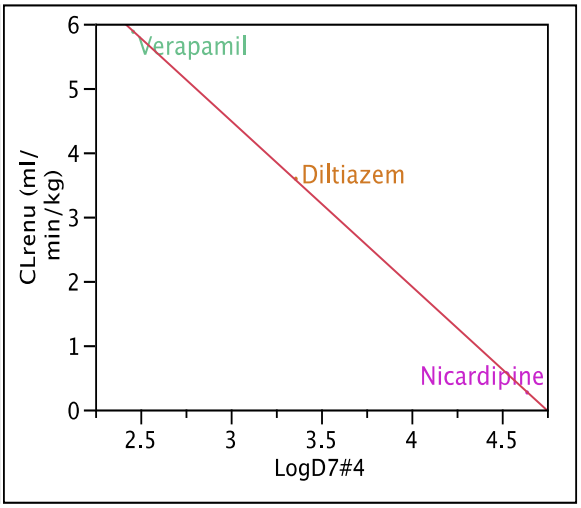


Figure 4.34 CL_{ren}^{u} vs. $logD_{7.4}$ (without amlodipine)

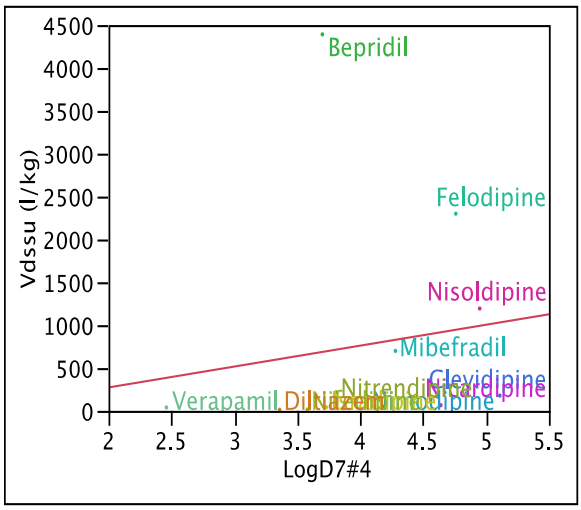


Figure 4.35 Vd_{ss}^{u} vs. $logD_{7.4}$ (without amlodipine)

Generally, most CCB in this dataset are highly lipophilic, hepatically metabolized compounds.[25, 6] Thus, CL_{nonren} primarily represents their hepatic intrinsic clearance and PPB. CL_{tot}^{u} and CL_{nonren}^{u} showed a strong relationship with $logD_{7.4}$; as $logD_{7.4}$ increased, CL_{tot}^{u} and CL_{nonren}^{u} increased. CCB have very low renal clearance, and, if these drugs have low or negligible renal clearances, they are most likely to be dependent on hepatic metabolism for clearance.[47] Smith et al.[48] has studied the relationship for a series of compounds and found that, where increasing $logD_{7.4}$ above zero shows a significant

reduction in CL_{ren}; however, increasing lipophilicity results in higher metabolic clearance. For CCB, the expected trend was found between $logD_{7.4}$ and renal clearance, but it was not significant which may have been due to small sample size.

Lipophilicity is an important property in governing binding of drugs to the active site of many enzymes of drug metabolism and also portioning into the liver. Thus, while increasing lipophilicity ($logD_{7.4}$) above zero will reduce renal clearance, it may increase metabolic clearance. Therefore, there were two opposing effects; unbound renal clearance decreasing with $logD_{7.4}$ and CL_{nonren}^{u} increasing with log $D_{7.4}$. The two main factors that affect the CL_{nonren} are PPB and/or intrinsic clearance.[17] There was a significant negative relationship between log $D_{7.4}$ and f_u, and there were no significant relationships between log $D_{7.4}$ and both CL_{nonren} and CL_{tot}, but these relationships became significant after correcting of plasma protein binding. As discussed before $logD_{7.4}$ was found to lead to increase hepatic CL_{int} (smith et al.[40]).which explains the relationship for CL_{nonren}^{u}. Overall, the decrease in f_u with $logD_{7.4}$, is therefore offset by the increase in CL_{int} leading to a lack of effects on CL_{nonren}.

Table 4.8 shows a comparison of the univariate effects of $logD_{7.4}$ on the human PK variables for opioids[21], β-ARLs[21], benzodiazepines[45] and CCB (without amlodipine):

$LogD_{7.4}$ was found to be the most important molecular property to be associated with biological relevant PK properties when compared to the three groups, versus $logD_{7.4}$ especially for lipophilic compounds like β-ARLs and opioids.[21] However, the slope of f_u is shallower for CCB, but it still has the same, expected direction as the other drug classes. When $logD_{7.4}$ increases, f_u decreases, however, the slope is slower here compared to other groups because the number of compounds that was used in this study is lower

than the other groups and because there were two outliers (diltiazem and verapamil) in f_u. Another important reason is that CCB are highly PPB, and the fraction unbound in plasma is very low in most of these compounds. In fact, the mean values of f_u are 3.5%, 47%, and 53% for CCB, opioids, and β-ARLs respectively.[21] Finally, CCB have the lowest range in $logD_{7.4}$ compared to the other drug classes.

The relationship between CL_{tot}^{u} and $logD_{7.4}$ has a much steeper slope for CCB compared to the other drug classes. The values of $logD_{7.4}$ indicate that most of the CCB in this dataset are highly lipophilic drugs while in the other groups both hydrophilic and lipophilic drugs are included. Therefore, the slopes obtained in the univariate log-linear regression between CL_{tot}^{u} and $logD_{7.4}$ is steeper for CCB than those obtained for the other three groups. After correcting for renal clearance, to focus on metabolic clearance, better sensitivity can be seen between CL_{tot}^{u} and $logD_{7.4}$ compared with CL_{tot}^{u}; the slope/sensitivity values also become more consistent with the other drug classes. A possible explanation for this finding is that, because CCB are mainly metabolized by cytochrome P-450(3A) in the liver and since CCB have an average, higher $logD_{7.4}$ values, they become more vulnerable to cytochrome P-450(3A) metabolism, thus leading to a higher hepatic clearance .[23, 24] There is a positive relationship between $logD_{7.4}$ and CL_{int}, and hence these compounds are mainly eliminated by metabolism so they are very sensitive to any change in $logD_{7.4}$. On the other hand, in the other drug classes, like β-ARLs, there are some drugs, such as atenolol and sotalol, which are mainly cleared by the renal pathways, and many factors besides $logD_{7.4}$ may affect their overall elimination.[36]

Univariate analysis showed a significant effect of HBA, clogP and $logD_{7.4}$ on certain human PK properties. However, $logD_{7.4}$ ranges from 1.5 to 5, was the most

important molecular property, and affected most of the biological relevant PK properties for CCB. The slopes for all PK variables vs. log $logD_{7.4}$ remained similar among the dataset with and without amlodipine, thus demonstrating that the effect of PC on these variables was similar. However, exclusion of amlodipine lead to improvement in r^2, and a decrease in p-value, conforming that amlodipine is a PK outlier, whose Vd_{ss} and f_u.in particular, do not follow the trend of $logD_{7.4.}$

There were a number of limitations in this study: the first limitation was that the studies used to obtain the PK values did not have the same study design. The studies varied from drug to drug with regard to administered dose, blood sampling times, bioanalytical techniques, the number of subjects, etc. The second limitation was that the PK values reported were mean values and did not account for inter-subject variability. Furthermore, CL_{ren} for most of the compounds was poorly estimated and/or not available since most of the compounds are eliminated by nonrenal pathways. A further limitation was that the final sample size of CCB used in this study was relatively small, and, more importantly, the sample was limited to drugs that made it through the drug development to clinical use. Thus, the final database is representative of the clinically used CCB, but not covering a large range of PC properties (other than $logD_{7.4}$), as would have been desirable in order to establish generalizable QSPKR. In addition, the non-dihydropyridine class of CCB contained only a limited number of compounds, which made it difficult to compare within the dihydropyridine group. Finally, due to the small sample size and limited diversity in PC variables (except $logD_{7.4}$) only linear, univariate relationships were explored, other nonlinear models and/or interactions between more than one PC variable were not studied.

4.1.4 Overall conclusions

CCB in the final dataset (n=14) are – based on their PC characteristics - a fairly homogeneous, but clinically representative group of compounds that are highly lipophilic ($logD_{7.4}$>3) for most of the compounds except amlodipine ($logD_{7.4}$ =1.5), which makes them more vulnerable to hepatic cytochrome P-450(3A) metabolism, leading to high hepatic/nonrenal clearance, while f_u and CL_{ren} are low. The final QSPKR obtained in this study showed significant and plausible relationships between biological relevant PK properties and $logD_{7.4}$; the observed effects were consistent with other classes of drugs previously studied, i.e., opioids, β-ARL and benzodiazepines. However, slope estimates for the relationships of CL_{nonren}^{u} and CL_{tot}^{u} as a function of $logD_{7.4}$ for CCB were higher compared to these other compounds, most likely as a result of the higher average lipophilicity of CCB. However, amlodipine proved to be an outlier based on its high HBD (3), lowest $logD_{7.4}$ (1.5) value, which despite the overall trends, led to the highest Vd_{ss} (20 l/kg), the second lowest CL_{tot} value (6.9 ml/min/kg) and relatively high PPB (f_u=2%). This reflects presumably the effects of increased basicity of the amlodipine molecule, not captured in the PC variables. Better understanding of the PK and PD properties of amlodipine may make an important contribution to the discovery of new drugs or the development of the existing CCB.

Table 4.6 Regressions between PC and PK Variables (all CCB)

	f_u	log (Vd_{ss})	log (CL_{tot})	log (CL_{ren})	log (CL_{nonren})	log (CL_{tot}^u)	log (CL_{ren}^u)	log (CL_{nonren}^u)	Log (Vd_{ss}^u)
MW	n=13 r^2<0.001 Slope <0.001 (N.S.)	n=14 r^2=0.12 Slope = -0.003 (N.S.)	n=14 r^2= <0.001 slope< -0.001 (N.S.)	n= 4 r^2= 0.56 slope= -0.025 (N.S.)	n=9 r^2=0.08 slope= 0.002 (N.S.)	n=13 r^2<0.001 slope <0.001 (N.S.)	n=4 r^2=0.64 slope= -0.018 (N.S.)	n=9 r^2=0.023 slope= 0.004 (N.S.)	n=13 r^2=0.006 slope= -0.001 (N.S.)
$logD_{7.4}$	n=13 r^2=0.15 Slope = -0.02 (N.S.)	n=14 r^2=0.25 slope= -0.25 (N.S.)	n=14 r^2=0.06 slope= 0.12 (N.S.)	n=4 r^2=0.52 slope= -0.615 (N.S.)	n=9 r^2=0.24 slope= 0.15 (N.S.)	**n=13** **r^2=0.36** **slope=** **0.53** **(p=0.03)**	n=4 r^2=0.89 slope= -0.52 (p=0.053)	n=9 r^2=0.42 slope= 0.56 (p=0.057)	n=13 r^2=0.03 slope= 0.14 (N.S.)
nRot	n=13 r^2=0.01 slope= -0.002	n=14 r^2=0.00 slope= 0.01	n=14 r^2=0.01 slope= -0.012	n=4 r^2=0.03 slope= -0.085	n=9 r^2=0.01 slope= 0.019	n=13 r^2<0.001 slope= <0.001	n=4 r^2<0.001 slope= 0.007	n=9 r^2=0.001 slope= -0.015	n=13 r^2=0.02 slope= 0.05

	(N.S.)	(N.S.)	(N.S.)	(N.S.)	(N.S.)	(N.S.)	(N.S.)	(N.S.)	(N.S.)
HBA	n=13 r^2=0.06 slope= -0.007 (N.S.)	**n=14** **r^2=0.37** **Slope =** **-0.131** **P(0.02)**	n=14 r^2=0.01 Slope =0.0146 (N.S.)	n=4 r^2=0.88 Slope = -0.622 p(0.06)	n=9 r^2=0 Slope = 0 (N.S.)	n=13 r^2=0.02 Slope = -0.07 (N.S.)	n=4 r^2=0.5 Slope = -0.31 (N.S.)	n=9 r^2=0.007 Slope = 0.0625 (N.S.)	n=13 r^2=0.19 Slope = -0.202 (N.S.)
HBD	n=13 r^2=0.02 Slope = -0.010 (N.S.)	n=14 r^2=0.05 Slope = 0.16 (N.S.)	n=14 r^2=0.03 Slope = -0.08 (N.S.)	n=4 r^2<0.001 Slope = -0.002 (N.S.)	n=9 r^2=0.1 Slope = -0.15 (N.S.)	n=13 r^2=<0.001 Slope = <-0.001 (N.S.)	n=4 r^2=0.11 Slope = 0.19 (N.S.)	n=9 r^2=0.02 Slope = 0.181 (N.S.)	n=13 r^2=0.012 Slope = 0.13 (N.S.)
clogP	n=13 r^2=0.25 Slope = -0.03 (0.08)	n=14 r^2<0.01 Slope = 0.006 (N.S.)	n=14 r^2<0.001 Slope = 0.005 (N.S.)	n=4 r2=0.64 Slope = -1.09 (N.S.)	n=9 r^2=0.28 Slope = 0.26 (N.S.)	**n=13** **r^2=0.32** **Slope =** **0.55** **(0.04)**	n=4 r^2=0.7 Slope = -0.809 (N.S.)	**n=9** **r^2=0.53** **Slope =** **0.99** **(0.025)**	n=13 r^2=0.27 Slope = 0.47 (N.S.)

Table 4.7 Regressions between PC and PK Variables without Amlodipine

	f_u	log (Vd_{ss})	log (CL_{tot})	log (CL_{ren})	log (CL_{nonren})	log (CL_{tot}^u)	log (CL_{ren}^u)	log (CL_{nonren}^u)	log (Vd_{ss}^u)
MW	n=12 r^2<0.001 slope 0.001 (N.S.)	n=13 r^2=0.18 slope = -0.003 (N.S.)	n=13 r^2 <0.001 slope< -0.001 (N.S.)	n= 3 r^2= 0.66 slope= -0.033 (N.S.)	n=8 r^2=0.09 slope= 0.002 (N.S.)	n=12 r^2<0.001 slope <0.001 (N.S.)	n=3 r^2=0.49 slope= -0.016 (N.S.)	n=8 r^2=0.023 slope= 0.003 (N.S.)	n=12 r^2=0.006 slope= -0.001 (N.S.)
$logD_{7.4}$	**n=12** **r^2=0.4** **Slope =** **-0.04** **(0.027)**	n=13 r^2=0.08 slope= -0.16 (N.S.)	n=13 r^2=0.06 slope= 0.11 (N.S.)	n=3 r^2=0.8 slope= -1.11 (N.S.)	n=8 r^2=0.12 slope= 0.14 (N.S.)	**n=12** **r^2=0.61** **slope=** **0.97** **(p=0.002)**	**n=3** **r^2=0.91** **slope=** **-0.66** **(p=0.18)**	**n=8** **r^2=0.77** **slope=** **1.06** **(p=0.004)**	n=12 r^2=0.25 slope= 0.55 (N.S.)
nRot	n=12 r^2=0.006 slope= -0.019	n=13 r^2=0.01 slope= -0.014	n=13 r^2<0.001 slope= -0.003	n=3 r^2=0.04 slope= -0.09	n=8 r^2=0.07 slope= 0.04	n=12 r^2<0.001 slope= 0.004	n=3 r^2<0.003 slope= -0.014	n=8 r^2<0.001 slope= -0.010	n=12 r^2=0.009 slope= 0.03

	(N.S.)	(N.S.)	(N.S.)	(N.S.)	(N.S.)	(N.S.)	(N.S.)	(N.S.)	(N.S.)
HBA	n=12 r^2=0.06 slope= -0.007 (N.S.)	**n=13** **r^2=0.54** **slope** **=-0.138** **(0.004)**	n=13 r^2=0.014 slope =0.017 (N.S.)	n=3 r^2=0.95 slope = -0.63 (0.13)	n=8 r^2=0 slope = 0 (N.S.)	n=12 r^2=0.019 slope = -0.07 (N.S.)	n=3 r^2=0.86 slope = -0.33 (N.S.)	n=8 r^2=0.007 slope = 0.0625 (N.S.)	n=12 r^2=0.21 slope = -0.205 (N.S.)
HBD	n=12 r^2=0.013 Slope = -0.017 (N.S.)	n=13 r^2=0.14 Slope = -0.45 (N.S.)	n=13 r^2=0.012 Slope = 0.104 (N.S.)	n=3 r^2=0.22 Slope = -1.1 (N.S.)	n=8 r^2<0.001 Slope = -0.02 (N.S.)	n=12 r^2=0.006 Slope = 0.2 (N.S.)	n=3 r2=0.37 Slope = -0.8 (N.S.)	n=8 r^2=0.16 Slope =1.2 (N.S.)	n=12 r^2=0.04 Slope = -0.45 (N.S.)
clogP	**n=12** **r2=0.36** **Slope =** **-0.04** **(0.03)**	n=13 r^2<0.08 Slope = 0.15 (N.S.)	n=13 r^2<0.01 Slope = -0.04 (N.S.)	n=3 r^2=0.84 Slope = -1.6 (0.25)	n=8 r^2=0.18 Slope = 0.24 (N.S.)	**n=12** **r^2=0.36** **Slope =** **0.66** **(0.03)**	n=3 r^2=0.71 Slope = -0.84 (N.S.)	**n=8** **r^2=0.72** **Slope =** **1.40** **(0.0069)**	**n=12** **r^2=0.54** **Slope =** **0.72** **(0.005)**

Table 4.8 Comparison between CCB and other drug classes as a function of $logD_{7.4}$

Class of Compounds	**f_u [%]**	**Log Vd_{ss}^u [l/kg]**	**Log CL_{tot}^u [ml/min/kg]**	**Log CL_{ren}^u [ml/min/kg]**	**Log CL_{nonren}^u [ml/min/kg]**
Opioids[21]	**n = 20**	**n = 19**	**n = 20**	n = 10	**n = 10**
	r^2 = 0.49	**r^2 = 0.50**	**r^2 = 0.35**	r^2 = 0.02	**r^2 = 0.88**
	Slope = -0.13	**Slope = 0.30**	**Slope = 0.20**	Slope = 0.01	**Slope = 0.34**
	p < 0.05	**p < 0.05**	**p < 0.05**	N.S	**p < 0.05**
β- ARLs[21]	**n = 30**	**n = 30**	**n = 30**	n = 26	**n = 26**
	r^2 = 0.64	**r^2 = 0.77**	**r^2 = 0.66**	r^2 = 0.05	**r^2 = 0.51**
	Slope = -0.22	**Slope = 0.45**	**Slope = 0.42**	Slope = -0.09	**Slope = 0.52**
	p < 0.05	**p < 0.05**	**p < 0.05**	N.S	**p < 0.05**
Benzodiazepines[45]	**n = 17**	**n = 16**	n = 17	n = 8	**n = 8**
	r^2 = 0.51	**r^2 = 0.57**	r^2 = 0.19	r^2 = 0.23	**r^2 = 0.81**
	Slope = -0.20	**Slope = 0.60**	Slope = 0.28	Slope = -0.37	**Slope = 0.66**
	p < 0.05	**p < 0.05**	N.S	N.S	**p < 0.05**
CCB (without Amlodipine)	**n = 12**	n = 12	**n = 12**	n = 3	**n = 8**
	r^2 = 0.40	**r^2 = 0.25**	**r^2 = 0.61**	r^2 = 0.91	**r^2 = 0.77**
	Slope = -0.04	Slope = 0.55	**Slope = 0.97**	Slope = -0.66	**Slope = 1.06**
	p < 0.027	N.S	**p<0.002**	N.S	**(p=0.004)**

Appendix I

Summary of PK variables across studies

(final values including ± SD for $CL_{tot,}$ and Vd_{ss})

Amlodipine

Reference	Sample size (Age)	BW (kg)	Dose (mg/kg)	Duration (min)	Sampling Schedule (hrs)	Assay	**LLOQ** Plasma	PK Analysis	PK endpoints		
					Plasma		ng/ml		C_{max} (ng/ml)	CL_{tot} (ml/min/kg)	Vd_{ss} (l/kg)
Stopher et al (88)[49]	H n=2	70	0.07	Bolus	0-250	GC-ECD	0.2	NCA*	27	7	19.74
Faulkner et al (86)[50]	H n=12 (26)	66.6	0.15	10	0.1-144	GC-ECD	0.2	NCA*	40	6.89	20.55
Vincent et al (00)[51]	H n=20 (32)	70	0.14	10	0-216	GC-ECD	0.2	NCA*	34.8	6.93	21
Final values including ± SD (n=) for $CL_{tot,}$ and Vd										**6.94±0.06 (n=3)**	**20.43±0.64 (n=3)**

(**a**) Assume 70 kg as body weight, (*) calculated using graph

Bepridil

Reference	Sample size (Age)	BW (kg)	Dose (mg/kg)	Duration (min)	Sampling Schedule (hrs)	Assay	LLOQ Plasma	PK Analysis	PK endpoints				
					Plasma		ng/ml		C_{max} (ng/ml)	AUC (ng*min/ml)	CL_{tot} (ml/min/kg)	Vd_{ss} (l/kg)	$t_{1/2}$ (h)
Lesko et al (86)[54]	P[b] n=16 (55)	75	0.05	30	0-24	HPLC	5	NCA	2478	5354	8.7	10.1	14.9

(**b**) Post-operative patient with normal function liver and kidney

Clevidipine

Reference	Sample size (Age)	BW (kg)	Dose (mg/kg)	Duration (min)	Sampling Schedule (hrs)	Assay	LLOQ Plasma	PK Analysis	PK endpoints		
					Plasma		ng/ml		C_{max} (ng/ml)	CL_{tot} (ml/min/kg)	Vd_{ss} (l/kg)
Ericsson et al (99)[56]	H 25 (29)	75	0.001-0.44		0.75	GC-MS	2.2	Compart		121	0.56
Ericsson et al (99)[57]	H 12 (26)	66.6	0.36	60	0-1.5	GC-MS	0.46	Compart	37	142	0.58
Final values including ± SD (n=) for $CL_{tot,}$ and Vd										**131.50±14.8 (n=2).**	**0.57±0.01 (n=2)**

Diltiazem

Reference	Sample size (Age)	BW (kg)	Dose (mg/kg)	Duration (min)	Sampling Schedule (hrs)	Assay	LLOQ Plasma	PK Analysis	PK endpoints		
					Plasma		ng/ml		C_{max} (ng/ml)	CL_{tot} (ml/min/kg)	Vd_{ss} (l/kg)
Smith et al(1983)[59]	H n=8 (22-58)	84	0.24	10	0-48	HPLC	10	Compart	294	10.4	3.18
Bianchetti et al (95)[60]	H n=24 (25.5)	72.4	0.30	30	0-36	GC-MS.	5	Compart	168	9.2±1.2	1.94
Tawashi (91)[61]	H n=3 (27.3)	69.8	0.29	5	0-48	HPLC-UV	2.5	Compart	124	12±2	
								NCA	124	12.6	3.21
Ochs et al(1984)[62]	H n=6 (25)	70	0.28	20	0-36	GC ELD	5	NCA*	179	11.26	2.51
Final values including ± SD (n=) for $CL_{tot,}$ and Vd										**10.87±1.4(n=4)**	**2.7±0.6(n=4)**

(*) calculated using graph

Felodipine

Reference	Sample size (Age)	BW (kg)	Dose (mg/kg)	Duration (min)	Sampling Schedule (hrs)	Assay	LLOQ Plasma	PK Analysis	PK endpoints		
					Plasma		ng/ml		C_{max} (ng/ml)	CL_{tot} (ml/min/kg)	Vd_{ss} (l/kg)
Edgar et al(1985)[64]	H n=8 (25 y)	74	0.034	30	0-30	GC W/ ELD	0.76	Compart	25.6	11.1	
								NCA	25.6	6.4	5.8
Lundahl et al(1997)[65]	H n=12 (25)	75	0.02	60	0-24	GC W/ ELD	0.76	Compart	12.5	11.2	NA
Edgar et al(1987)[66]	H n=10 26	73	0.014	20	0-10	GC W/ ELD	0.76	Compart	12.38	14.9	NA
Edgar et al(1987)[66]	H n=10 (26y)	73	0.04	20	0-10	GC W/ ELD	0.76	Compart	37.05	14.3	2.93(cal)
Landehal (1988)[67]	H n=12 (26)	75	0.0005	5	0-27	HPLC-l sci.	0.9	NCA	2	12.5	10.3±3.4
Final values including ± SD (n=) for $CL_{tot,}$ and Vd										**11.86±3.4(n=5)**	**8.05±3.7(n=3)**

(a) Assume 70 kg as body weight, (*) calculated using graph

Fendiline

Reference	Sample size (Age)	BW (kg)	Dose (mg/kg)	Duration (min)	Sampling Schedule (hrs)	Assay	LLOQ Plasma	PK Analysis	PK endpoints				
					Plasma		ng/ml		C_{max} (ng/ml)	AUC (ng*min/ml)	CL_{tot} (ml/min/kg)	Vd_{ss} (l/kg)	$t_{1/2}$ (h)
Kukovetz et al (82)[69]	H n=6 (23)	66	0.05	Bolus	0-48	TLC	5	NCA*	4.2	2616	17.4	13.8	10.55

Isradipine

Reference	Sample size (Age)	BW (kg)	Dose (mg/kg)	Duration (min)	Sampling Schedule (hrs)	Assay	LLOQ Plasma	PK Analysis	PK endpoints		
					Plasma		ng/ml		C_{max} (ng/ml)	CL_{tot} (ml/min/kg)	Vd_{ss} (l/kg)
Christensen et al (2000)[70]	H n=10 (24.2)	71.9	(0.026)	1mg/60m & 0.9mg/3h	0-10	GC-NSD	0.2	NCA*	7	15.6	1.43
Carrara et al (1994)[71]	H n=10	72±11	(0.014)	5	0-24	RIA	0.03	NCA*	128	5.84	0.65
Cotting et al (1990)[72]	H n=7 (43)	70	0.014	10	0-28	GC-MS	0.2	Compart	NA	15.5±3	NA
Final values including ± SD (n=) for $CL_{tot,}$ and Vd										**12.3 ±5.6(n=3)**	**1.04 ±0.5(n=3)**

Radioimmuno Assay (RIA), (**a**) Assume 70 kg as body weight, (*) calculated using graph

Mibefradil

Reference	Sample size (Age)	BW (kg)	Dose (mg/kg)	Duration (min)	Sampling Schedule (hrs)	Assay	LLOQ Plasma	PK Analysis	PK endpoints		
					Plasma		ng/ml		C_{max} (ng/ml)	CL_{tot} (ml/min/kg)	Vd_{ss} (l/kg)
Welker et al (98)[74]	H n=18 (26.5)	70	0.28	30	0-250	HPLC	2	NCA	110	4.07	2.8
			0.57							3.44	3.0
			1.14							3.58	2.5
Final values including ± SD (n=) for $CL_{tot,}$ and Vd										**3.7±0.3(n=5)**	**2.7 ±0.3(n=3)**

(**a**) Assume 70 kg as body weight, (*) calculated using graph

Nicardipine

Reference	Sample size (Age)	BW (kg)	Dose (mg/kg)	Duration (min)	Sampling Schedule (hrs)	Assay	LLOQ Plasma	PK Analysis	PK endpoints		
					Plasma		ng/ml		C_{max} (ng/ml)	CL_{tot} (ml/min/kg)	Vd_{ss} (l/kg)
Graham et al(85)[76]	H n=5	70	0.21	180	0-12	GC, HPLC	2-3	Compart	121	8.23	
								NCA*	121	8.12	0.68
Guerret et al (89)[77]	H n=6	72	0.02	5	0-12	GC-MS	0.05	Compart		11.3±0.98	1±0.16
Higuci et al (80)[78]	H n=2	59	0.01	5	0-2	GC W/ ELD	2-3	NCA	24	7	
								NCA*	24	8.15	0.54
Higuci et al (80)[78]	H n=2	59	0.02	5	0-2	GC W/ ELD	2-3	NCA	74	9	
								NCA*	74	8.22	0.45
Campbell et al(85)[79]	H n=14 (30)	70[a]	0.16	1	0-6	HPLC/GC-ECD	5	Compart	160	17	0.89
Final values including ± SD (n=) for $CL_{tot,}$ and Vd										**10.56±3.8 (n=5)**	**0.71 ±0.23 (n=5)**

(**a**) Assume 70 kg as body weight, (*) calculated using graph

Nifedipine

Reference	Sample size (Age)	BW (kg)	Dose (mg/kg)	Duration (min)	Sampling Schedule (hrs)	Assay	LLOQ Plasma	PK Analysis	PK endpoints		
					Plasma		ng/ml		C_{max} (ng/ml)	CL_{tot} (ml/min/kg)	Vd_{ss} (l/kg)
Foster et al(83)[81]	H n=9 (25)	75.3	0.013	5	-50m to + 30 hr	GC W/ ELD	1	NCA	25.97± 4.81	10.33±1.5	1.12± 0.14
Ramsch et al(86)[82]	H n=7	70[a]	0.024	4-5	0-8	GC W/ ELD	1	Compart	102	7.6±4	0.47
Kleinbloesem (85)[83]	H n=5 (44)	80	0.056	45	0-8	HPLC-UV	5	Compart	118	6.8±1.6	0.78±0.2.3
Rashid et al (95)[84]	H n=8 cau (22)	74	0.033	2.5	0-12	HPLC-UV	3	NCA	92	8.5	0.97
Bortel et al(89)[85]	H n=6 (44)	75.5	0.058	60	0-8	HPLC-UV	3	NCA	NA	6.47	NA
Robertson et al (88)[86]	H n=5 (27)	75	0.033	5	0-6	HPLC-UV	3	NCA	110	6.92	0.76
Kleinbloesem (84)[87]	H n=5 (42)	75	0.06	50	0-7	HPLC-UV	2	Compart	118	6.8±1.6	0.78±0.2.3
Waller (84)[88]	H n=7 (26)	75	0.046	4	0-8	HPLC-UV	3	NCA*	245	6.1	0.71
Final values including ± SD (n=) for $CL_{tot,}$ and Vd										**7.44±1.3 (n=8)**	**0.66 ±0.3 (n=7)**

Nimodipine

Reference	Sample size (Age)	BW (kg)	Dose (mg/kg)	Duration (min)	Sampling Schedule (hrs)	Assay	LLOQ Plasma	PK Analysis	PK endpoints		
					Plasma		ng/ml		C_{max} (ng/ml)	CL_{tot} (ml/min/kg)	Vd_{ss} (l/kg)
Ramsch et al (86)[82]	H n=6	70[a]	0.03	4-5	0-6	GC W/ ELD	0.5	Compart	92	14±4	0.94±0.41
Ramsch et al (86)[82]	H n=2	70[a]	0.34	24 h	0-30	HPLC	0.5	Compart	12	18.7	2.3
Mck et al (96)[89]	H n=24 (30)	66.4	0.015	60	0-24	GC-ECD	0.1	NCA	11.34	16.6	1.21
Final values including ± SD (n=) for $CL_{tot,}$ and Vd										**16.4±2.35 (n=3)**	**1.48 ±0.7 (n=3)**

(**a**) Assume 70 kg as body weight, (*) calculated using graph

Nisoldipine

Reference	Sample size (Age)	BW (kg)	Dose (mg/kg)	Duration (min)	Sampling Schedule (hrs)	Assay	LLOQ Plasma	PK Analysis	PK endpoints		
					Plasma		ng/ml		C_{max} (ng/ml)	CL_{tot} (ml/min/kg)	Vd_{ss} (l/kg)
Van harten et al (88)[91]	8H(54)	74	0.374	40	0-32	GC ELD	0.25	NCA	5.2±1.1	11.4±4.1	4.1±2.1
Van harten et al (89)[92]	6 Hypertension (62)	87	0.022	120	0-24	GC ELD	0.25	NCA	5.7	11.7	5.9±1.8
Ahr et al (1987)[93]	4H	70[a]	0.08	1200	0-44	GC-MS/ECD	0.2	Compart	6.22±1	11.3±1.8	2.7±1.2
Final values including ± SD (n=) for $CL_{tot,}$ and Vd										**11.3±0.37 (n=3)**	**3.6 ±1.9 (n=3)**

Nitrendipine

Reference	Sample size (Age)	BW (kg)	Dose (mg/kg)	Duration (min)	Sampling Schedule (hrs)	Assay	LLOQ Plasma	PK Analysis	PK endpoints			
					Plasma		ng/ml		C_{max} (ng/ml)	CL_{tot} (ml/min/kg)	Vd_{ss} (l/kg)	
Ramsch et al (86)[82]	H n=9	70	0.04	3-5	0-6	GC W/ ELD	0.5	Compart	9.7	34.5±16.6	6.6±5.5	
Mikus et al (87)[96]	H n=6 (27)	70	0.028	30	0-25.5	CG-SIM	0.1	Compart	10.97	18.65±3.59	5.39±2.35	
Dylewicz et al (87)[97]	H n=6	69.7	0.07	3-5	0-48	GC W/ ELD	0.5	Compart	14	18.5	NA	
Soon & Breimer (91)[98]	H n=9 (24)	74	0.04	4	0-12	GC-ECD	0.2	NCA& Compart	NA	21±3.4	3.79±1.32	
Graefe et al (88)[99]	H n=9 (25)	75	0.04	4	0-6	GC-ECD	0.5	NCA	NA	32.2(20-52)	3.6	
Final values including ± SD (n=) for $CL_{tot,}$ and Vd										**24.9±7.7 (n=5)**	**4.8 ±2.5 (n=4)**	

SIM: Single Ion Monitoring

Verapamil

Reference	Sample size (Age)	BW (kg)	Dose (mg/kg)	Duration (min)	Sampling Schedule (hrs)	Assay	LLOQ Plasma	PK Analysis	PK endpoints		
					Plasma		ng/ml		C_{max} (ng/ml)	CL_{tot} (ml/min/kg)	Vd_{ss} (l/kg)
Freedman et al(81)[100]	4healthy 2patients (47)	79	0.16	10-15	0-14	HPLC	2	Compart	200	10.94	3.4
Dominic et al(81)[101]	8H(26)	71	0.2	3-5	0 -6	GC with thermo -ionic det.	5	Compart	274	14.9±3.8	2.52
Abernethy et al(85)[102]	7(29) Hypertension P (no-med)	76	0.13	10	0-24	GC-NPD	2	Compart	180	15.5±4.5	4.3±1.1
Eichelbaum et al(81)[103]	6H(23)	86	0.15	5	0-13	mass fragmen-tography	2.2	Compart	31	19.67	6.15
Eichelbaum et al(84)[104]	5H(25)	69	0.14	5	0-12	mass fragmen-tography	2.2	Compart	270	14.27	3.4
Final values including ± SD (n=) for $CL_{tot,}$ and Vd										**15.1±3.1 (n=5)**	**4.0 ±1.4 (n=5)**

NPD=Nitrogen Phosphorous Detection, h=healthy volunteers, p= patient

Appendix II
Human PK Study summaries of CCB

Amlodipine

Reference	Sample size (Age)	BW (kg)	Dose (mg/kg)	Duration (min)	Sampling Schedule (hrs)	Assay	LLOQ Plasma	PK Analysis	PK endpoints				
					Plasma		ng/ml		C_{max} (ng/ml)	AUC (ng*min/ml)	CL_{tot} (ml/min/kg)	Vd_{ss} (l/kg)	$t_{1/2}$ (h)
Stopher et al (88)[49]	H n=2	70	0.07	Bolus	0-250	GC-ECD	0.2	NCA*	27	9445	7	19.74	35
Faulkner et al (86)[50]	H n=12 (26)	66.6	0.15	10	0.1-144	GC-ECD	0.2	NCA*	40	21802	6.89	20.55	40.65
Vincent et al (00)[51]	H n=20 (32)	70	0.14	10	0-216	GC-ECD	0.2	NCA*	34.8	21480	6.93	21	38.3

(a) Assume 70 kg as body weight, (*) calculated using graph

Plasma protein binding studies:

Reference	Range (ng/ml)	Method	Assay	Protein binding
Stopher et al (88)[52]	50	Equilibrium dialysis	Liquid Scintillation	98%

Urine excretion study:

- Beresford et al (1988)[53]
- Determination of amlodipine in urine indicated that 4% of the dose to the human volunteers was excreted unchanged via the kidneys during the 0-72 h period after dosing.
- This study overestimate f_e because the accumulative amount of urine was not collected for a long period of time (4-5 $t_{1/2}$) and they only collect for three days and then extrapolated
- Only two healthy volunteers were used in this study

Bepridil

Reference	Sample size (Age)	BW (kg)	Dose (mg/kg)	Duration (min)	Sampling Schedule (hrs)	Assay	LLOQ Plasma	PK Analysis	PK endpoints				
					Plasma		ng/ml		C_{max} (ng/ml)	AUC (ng*min/ml)	CL_{tot} (ml/min/kg)	Vd_{ss} (l/kg)	$t_{1/2}$ (h)
Lesko et al (86)[54]	P[b] n=16 (55)	75	0.05	30	0-24	HPLC	5	NCA	2478	5354	8.7	10.1	14.9

(b) Post-operative patient with normal function liver and kidney

Plasma protein binding studies:

Reference	Range (ng/ml)	Method	Assay	Protein binding
Pritchard et al (98)[55]	200-4000	Equilibrium dialysis	Liquid Scintillation	99.76%

Clevidipine

Reference	Sample size (Age)	BW (kg)	Dose (mg/kg)	Duration (min)	Sampling Schedule (hrs)	Assay	LLOQ Plasma	PK Analysis	PK endpoints				
					Plasma		ng/ml		C_{max} (ng/ml)	AUC (ng*min/ml)	CL_{tot} (ml/min/kg)	Vd_{ss} (l/kg)	$t_{1/2}$ (h)
Ericsson et al (99)[56]	H 25 (29)	75	0.001-0.44		0.75	GC-MS	2.2	Compart			121	0.56	0.15
Ericsson et al (99)[57]	H 12 (26)	66.6	0.36	60	0-1.5	GC-MS	0.46	Compart	37	2535	142	0.58	0.25

Plasma protein binding studies:

Reference	Range (ng/ml)	Method	Protein binding
Ericsson et al (99)[58]	11.4-114	HPLC	99.68%

Urine excretion study:

- Ericsson et al (99)[57]
- Samples of urine were collected pre-dose and up to 168 h after the start of the clevidipine infusion. The sampling intervals were 24 h, except for the first investigational day, when urine was collected during intervals of 0-3, 3-6, 6-12 h and 12- 24 h after start of infusion.
- No parent drug was found in the urine.

Diltiazem

Reference	Sample size (Age)	BW (kg)	Dose (mg/kg)	Duration (min)	Sampling Schedule (hrs)	Assay	LLOQ Plasma	PK Analysis	PK endpoints				
					Plasma		ng/ml		C_{max} (ng/ml)	AUC (ng*min/ml)	CL_{tot} (ml/min/kg)	Vd_{ss} (l/kg)	$t_{1/2}$ (h)
Smith et al(1983)[59]	H n=8 (22-58)	84	0.24	10	0-48	HPLC	10	Compart	294	21000	10.4	3.18	4.45±1.5
Bianchetti et al (95)[60]	H n=24 (25.5)	72.4	0.30	30	0-36	GC-MS.	5	Compart	168	33660±3720	9.2±1.2	1.94	3.6±0.3
Tawashi (91)[61]	H n=3 (27.3)	69.8	0.29	5	0-48	HPLC-UV	2.5	Compart	124	24480±4020	12±2		2.7±0.41
								NCA	124	22710	12.6	3.21	4.44
Ochs et al(1984)[62]	H n=6 (25)	70	0.28	20	0-36	GC ELD	5	NCA	179	24844	11.5±0.72		11.2
								NCA*	179	23345	11.26	2.51	3.78
								Compart				5.2	

(*) calculated using graph

Plasma protein binding studies:

Reference	Range	Method	Assay	Protein binding
Bloedow et al (1985)[63]	30-2060 ng/ml	Equilibrium dialysis	Liquid scintillation	80

Urine excretion study:

- Tawashi et al (1991)[61]
- Urine collection (0-6, 6-12, 12-24, and 24-48 h)
- The unchanged drug in the urine was 6.6% (CL_{ren}=53 ml/min)

Felodipine

Reference	Sample size (Age)	BW (kg)	Dose (mg/kg)	Duration (min)	Sampling Schedule (hrs)	Assay	LLOQ Plasma	PK Analysis	PK endpoints				
					Plasma		ng/ml		C_{max} (ng/ml)	AUC (ng*min/ml)	CL_{tot} (ml/min/kg)	Vd_{ss} (l/kg)	$t_{1/2}$ (h)
Edgar et al(1985)[64]	H n=8 (25 y)	74	0.034	30	0-30	GC W/ ELD	0.76	Compart	25.6	3050	11.1		10.21
								NCA	25.6	5272	6.4	5.8	10.37
Lundahl et al(1997)[65]	H n=12 (25)	75	0.02	60	0-24	GC W/ ELD	0.76	Compart	12.5	1890	11.2	NA	2.9
Edgar et al(1987)[66]	H n=10 26	73	0.014	20	0-10	GC W/ ELD	0.76	Compart	12.38	934	14.9	NA	NA
Edgar et al(1987)[66]	H n=10 (26y)	73	0.04	20	0-10	GC W/ ELD	0.76	Compart	37.05	2886	14.3	2.93(cal)	NA
Landehal (1988)[67]	H n=12 (26)	75	0.0005	5	0-27	HPLC-l sci.	0.9	NCA	2	50.7	12.5	10.3±3.4	NA

(c) Assume 70 kg as body weight, (*) calculated using graph

Plasma protein binding studies:

Reference	Range (ng/ml)	Method	Assay	Protein binding
Valle et al(96)[68]	1.15-25	Ultrafiltration	Liquid scintillation	99.65%
Ericsson et al(99)[58]	9.6		Liquid Chromatography	99.60%

Urine excretion study:

- Edgar et al (1985)[64]
- Urine was quantitatively collected for 72 hours at the following times: blank, 0 to 4, 4 to 8, 8 to 12, 12 to 24, 24 to 36, 36 to 48, 48 to 60, and 60 to 72 hours. The unchanged drug in the urine was not observed.

Fendiline

Reference	Sample size (Age)	BW (kg)	Dose (mg/kg)	Duration (min)	Sampling Schedule (hrs)	Assay	LLOQ Plasma	PK Analysis	PK endpoints				
					Plasma		ng/ml		C_{max} (ng/ml)	AUC (ng*min/ml)	CL_{tot} (ml/min/kg)	Vd_{ss} (l/kg)	$t_{1/2}$ (h)
Kukovetz et al (82)[69]	H n=6 (23)	66	0.05	Bolus	0-48	TLC	5	NCA*	4.2	2616	17.4	13.8	10.55

Isradipine

Reference	Sample size (Age)	BW (kg)	Dose (mg/kg)	Duration (min)	Sampling Schedule (hrs)	Assay	LLOQ Plasma	PK Analysis	PK endpoints				
					Plasma		ng/ml		C_{max} (ng/ml)	AUC (ng*min/ml)	CL_{tot} (ml/min/kg)	Vd_{ss} (l/kg)	$t_{1/2}$ (h)
Christensen et al (2000)[70]	H n=10 (24.2)	71.9	(0.026)	1mg/60m & 0.9mg/3h	0-10	GC-NSD	0.2	NCA*	7	1694	15.6	1.43	1.33
Carrara et al (1994)[71]	H n=10	72±11	(0.014)	5	0-24	RIA	0.03	NCA*	128	2383	5.84	0.65	5.03
Cotting et al (1990)[72]	H n=7 (43)	70	0.014	10	0-28	GC-MS	0.2	Compart	NA	942±132	15.5±3	NA	5.1±2.4

Radioimmuno Assay (RIA), (**a**) Assume 70 kg as body weight, (*) calculated using graph

Plasma protein binding studies:

Reference	Range (ng/ml)	Method	Assay	Protein binding (%)
Pinquier et al (1988)[73]	(0.37-3.7)	Equilibrium dialysis	Liquid Scintillation	97.5

Mibefradil

Reference	Sample size (Age)	BW (kg)	Dose (mg/kg)	Duration (min)	Sampling Schedule (hrs)	Assay	LLOQ Plasma	PK Analysis	PK endpoints				
					Plasma		ng/ml		C_{max} (ng/ml)	AUC (ng*min/ml)	CL_{tot} (ml/min/kg)	Vd_{ss} (l/kg)	$t_{1/2}$ (h)
Welker et al (98)[74]	H n=18 (26.5)	70	0.28	30	0-250	HPLC	2	NCA	110	68796	4.07	2.8	12.3
			0.57							165697	3.44	3.0	14.8
			1.14							318435	3.58	2.5	10.3

(a) Assume 70 kg as body weight, (*) calculated using graph

Plasma protein binding studies:

Reference	Range (ng/ml)	Method	Assay	Protein binding
Horst & Welker (98)[75]	500-1000	Equilibrium dialysis	Liquid Scintillation	99.6%

Nicardipine

Reference	Sample size (Age)	BW (kg)	Dose (mg/kg)	Duration (min)	Sampling Schedule (hrs)	Assay	LLOQ Plasma	PK Analysis	PK endpoints				
					Plasma		ng/ml		C_{max} (ng/ml)	AUC (ng*min/ml)	CL_{tot} (ml/min/kg)	Vd_{ss} (l/kg)	$t_{1/2}$ (h)
Graham et al(85)[76]	H n=5	70	0.21	180	0-12	GC, HPLC	2-3	Compart	121	26037	8.23		4.75
								NCA*	121	26376	8.12	0.68	1.40
Guerret et al (89)[77]	H n=6	72	0.02	5	0-12	GC-MS	0.05	Compart		2280	11.3±0.98	1±0.16	4.1±1.6
Higuci et al (80)[78]	H n=2	59	0.01	5	0-2	GC W/ ELD	2-3	NCA	24	1400	7		1.05
								NCA*	24	1227	8.15	0.54	0.83
Higuci et al (80)[78]	H n=2	59	0.02	5	0-2	GC W/ ELD	2-3	NCA	74	2300	9		0.83
								NCA*	74	2433	8.22	0.45	0.83
Campbell et al(85)[79]	H n=14 (30)	70[a]	0.16	1	0-6	HPLC/GC-ECD	5	Compart	160	9411.7	17	0.89	0.97

(**a**) Assume 70 kg as body weight, (*) calculated using graph

Plasma protein binding studies:

Reference	Range (ng/ml)	Method	Assay	Protein binding (%)
Urien et al (1985)[80]	1000-40000	Equilibrium dialysis	Liquid scintillation	98.92

Urine excretion study:

- Garham et al (1985)[76]

The unchanged drug in the urine was not observed

Nifedipine

Reference	Sample size (Age)	BW (kg)	Dose (mg/kg)	Duration (min)	Sampling Schedule (hrs)	Assay	LLOQ Plasma	PK Analysis	PK endpoints				
					Plasma		ng/ml		C_{max} (ng/ml)	AUC (ng*min/ml)	CL_{tot} (ml/min/kg)	Vd_{ss} (l/kg)	$t_{1/2}$ (h)
Foster et al(83)[81]	H n=9 (25)	75.3	0.013	5	-50m to + 30 hr	GC W/ ELD	1	NCA	25.97± 4.81	1584.6± 223	10.33±1.5	1.12± 0.14	1.77±0.25
Ramsch et al(86)[82]	H n=7	70[a]	0.024	4-5	0-8	GC W/ ELD	1	Compart	102	3924±1824	7.6±4	0.47	2.5±2.4
Kleinbloesem (85)[83]	H n=5 (44)	80	0.056	45	0-8	HPLC-UV	5	Compart	118	8230	6.8±1.6	0.78±0.2.3	1.76±0.4
Rashid et al (95)[84]	H n=8 cau (22)	74	0.033	2.5	0-12	HPLC-UV	3	NCA	92	4440	8.5	0.97	1.7±0.5
Bortel et al(89)[85]	H n=6 (44)	75.5	0.058	60	0-8	HPLC-UV	3	NCA	NA	11000	6.47	NA	4.08±1.1
Robertson et al (88)[86]	H n=5 (27)	75	0.033	5	0-6	HPLC-UV	3	NCA	110	5040	6.92	0.76	1.9
Kleinbloesem (84)[87]	H n=5 (42)	75	0.06	50	0-7	HPLC-UV	2	Compart	118	8181±	6.8±1.6	0.78±0.2.3	1.76±0.4
Waller (84)[88]	H n=7 (26)	75	0.046	4	0-8	HPLC-UV	3	NCA*	245	7648	6.1	0.71	1.92

(**a**) Assume 70 kg as body weight, (*) calculated using graph

Plasma protein binding studies:

Reference	Range	Method	Assay	Protein binding (%)
Kleinbloesem (85)[83]	5-200ng/ml	Mem dialysis after centrifugation	Liquid scintillation	96
Bortel(89)[85]	NA	Equilibrium dialysis	Liquid scintillation	95.5

Urine excretion study:

- Ramach et al (1986)[82]
- Using Gas Chromatographic with ELD(5 ng/ml)
- The unchanged drug in the urine was not observed

Nimodipine

Reference	Sample size (Age)	BW (kg)	Dose (mg/kg)	Duration (min)	Sampling Schedule (hrs)	Assay	LLOQ Plasma	PK Analysis	PK endpoints				
					Plasma		ng/ml		C_{max} (ng/ml)	AUC (ng*min/ml)	CL_{tot} (ml/min/kg)	Vd_{ss} (l/kg)	$t_{1/2}$ (h)
Ramsch et al (86)[82]	H n=6	70[a]	0.03	4-5	0-6	GC W/ ELD	0.5	Compart	92	2328±798	14±4	0.94±0.41	1.1±0.2
Ramsch et al (86)[82]	H n=2	70[a]	0.34	24 h	0-30	HPLC	0.5	Compart	12	18181	18.7	2.3	1.5
Mck et al (96)[89]	H n=24 (30)	66.4	0.015	60	0-24	GC-ECD	0.1	NCA	11.34	960	16.6	1.21	1.4

(**a**) Assume 70 kg as body weight, (*) calculated using graph

Plasma protein binding studies:

- Ramach et al (1985)[90]

Plasma protein binding is 98%

Urine excretion study:

- Ramach et al (1985)[90]

The unchanged drug in the urine was not observed using HPLC

Nisoldipine

Reference	Sample size (Age)	BW (kg)	Dose (mg/kg)	Duration (min)	Sampling Schedule (hrs)	Assay	LLOQ Plasma	PK Analysis	PK endpoints				
					Plasma		ng/ml		C_{max} (ng/ml)	AUC (ng*min/ml)	CL_{tot} (ml/min/kg)	Vd_{ss} (l/kg)	$t_{1/2}$ (h)
Van harten et al (88)[91]	8H(54)	74	0.374	40	0-32	GC ELD	0.25	NCA	5.2±1.1	32807	11.4±4.1	4.1±2.1	9.7±5.4
Van harten et al (89)[92]	6 Hypertension (62)	87	0.022	120	0-24	GC ELD	0.25	NCA	5.7	1880	11.7	5.9±1.8	15.4±6.7
Ahr et al (1987)[93]	4H	70[a]	0.08	1200	0-44	GC-MS/ECD	0.2	Compart	6.22±1	7079	11.3±1.8	2.7±1.2	10.7±1.3

Plasma protein binding studies:

Reference	Range (ng/ml)	Method	Assay	Protein binding (%)
Boelaert et al (1988)[94]	100ng/ml	Ultrafiltration	LC	99.6±0.04
Ahr et al (1987)[93]	20-1000	Ultrafiltration	LC	99.7

Urine excretion study:

- Scherling et al (1987)[95]
- Urine collection: 0-4, 4-8, 8-12, 12-24 hr.
- The unchanged drug in the urine was not observed

Nitrendipine

Reference	Sample size (Age)	BW (kg)	Dose (mg/kg)	Duration (min)	Sampling Schedule (hrs)	Assay	LLOQ Plasma	PK Analysis	PK endpoints				
					Plasma		ng/ml		C_{max} (ng/ml)	AUC (ng*min/ml)	CL_{tot} (ml/min/kg)	Vd_{ss} (l/kg)	$t_{1/2}$ (h)
Ramsch et al (86)[82]	H n=9	70	0.04	3-5	0-6	GC W/ ELD	0.5	Compart	9.7	1512±1092	34.5±16.6	6.6±5.5	4.6±2.4
Mikus et al (87)[96]	H n=6 (27)	70	0.028	30	0-25.5	CG-SIM	0.1	Compart	10.97	1662±333.6	18.65±3.59	5.39±2.35	8.56±4.16
Dylewicz et al (87)[97]	H n=6	69.7	0.07	3-5	0-48	GC W/ ELD	0.5	Compart	14	3708±288	18.5	NA	2.19±0.45
Soon & Breimer (91)[98]	H n=9 (24)	74	0.04	4	0-12	GC-ECD	0.2	NCA& Compart	NA	1904	21±3.4	3.79±1.32	4±1.6
Graefe et al (88)[99]	H n=9 (25)	75	0.04	4	0-6	GC-ECD	0.5	NCA	NA	1242	32.2(20-52)	3.6	2.9

SIM: Single Ion Monitoring

Plasma protein binding studies:

Reference	Range	Method	Assay	Protein binding
Mikus et al (1987)[96]		Equilibrium dialysis	Liquid Scintillation	96.4-98.9
Dylewicz et al (1987)[97]		Equilibrium dialysis	Liquid Scintillation	97.9±0.4

Urine excretion study:

- Mikus et al (1987)[96]
- Urine collection: 0-4, 4-8, 8-12, 12-24, 24-48 hr.
- Less than 0.5% of the dose excreted as unchanged drug in urine
- Nitrendipine could not be detected in urine (Dylewicz et al (1987)[97]

Verapamil

Reference	Sample size (Age)	BW (kg)	Dose (mg/kg)	Duration (min)	Sampling Schedule (hrs)	Assay	LLOQ Plasma	PK Analysis	PK endpoints				
					Plasma		ng/ml		C_{max} (ng/ml)	AUC (ng*min/ml)	CL_{tot} (ml/min/kg)	Vd_{ss} (l/kg)	$t_{1/2}$ (h)
Freedman et al(81)[100]	4healthy 2patients (47)	79	0.16	10-15	0-14	HPLC	2	Compart	200	15060 ±3300	10.94	3.4	5
Dominic et al(81)[101]	8H(26)	71	0.2	3-5	0 -6	GC with thermo -ionic det.	5	Compart	274	13422.8	14.9±3.8	2.52	1.84± 0.4
Abernethy et al(85)[102]	7(29) Hypertension P (no-med)	76	0.13	10	0-24	GC-NPD	2	Compart	180	8520 ±2280	15.5±4.5	4.3±1.1	3.8±1.1
Eichelbaum et al(81)[103]	6H(23)	86	0.15	5	0-13	mass fragmen-tography	2.2	Compart	31	7949	19.67	6.15	3.69
Eichelbaum et al(84)[104]	5H(25)	69	0.14	5	0-12	mass fragmen-tography	2.2	Compart	270	10101	14.27	3.4	3.75

NPD=Nitrogen Phosphorous Detection, h=healthy volunteers, p= patient

Plasma protein binding studies:

Reference	Range ng/ml	Method	Assay	Protein binding
Schomerus et al(1976)[105]	50- 500	Equilibrium dialysis	Liquid scintillation	90.35%
Keefe et al(1980)[106]	35 -1,557	Equilibrium dialysis	Liquid scintillation	89.6 ± 0.17%

Urine excretion study:

- Toffoli et al (1987)[107]
- Urine collection: every 12 hr.
- The unchanged drug in the urine was 2%

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